New Precious Metal Compounds in Cancer Therapy

by

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DECLARATION

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Abstract

Cisplatin is one of the most effective cancer medications currently available. However, it is seriously limited by patient toxicity and drug resistance. As such, there is a real need for alternative treatments. Some compounds of gold(I) have been found to be biologically active in various contexts, including in killing cancer cells. The metal centres gold(III) and rhodium(II) are isoelectronic to the platinum(II) centre in cisplatin, and some of their compounds have been shown to have biological activity. The aims of this work were to prepare pyridine-containing complexes of the three metal centres gold(I), gold(III) and rhodium(I), and assess these complexes for *in vitro* anti-cancer activity.

Phenylpyridine and ferrocenylpyridine complexation was achieved with all three metal centres described above. With gold(I), either a chloride or pentafluorophenyl counter-anion was used. The rhodium(I) complexes contained 1,5-cyclooctadiene moieties linked to the metal centre via diene complexation, and a chloride counter-anion. Phenylpyridine complexes of gold(III) were achieved via standard reaction with tetrachloroaurate anion. However, the analogous ferrocenylpyridine complexes display unusually low stability and other unexpected physical properties, and are believed to be highly novel chloro-bridged gold dimers.

The 4-phenylpyridine complex of rhodium(I) was initially found to be active against cancer cells *in vitro*. It was, however, demonstrated that this activity was actually due to the breakdown product of this compound in DMSO. It was found that this breakdown product interacts with DNA, implying a similar mechanism of action to cisplatin. This is supported by the fact that a cisplatin-resistant cell line displays high cross-resistance against this product. (4-Phenylpyridine)gold(I) (pentafluorophenyl) was also found to be extremely active against cell lines *in vitro.*

Future work should include further studies on the ferrocenylpyridine complexes of gold(III), and the development of a more reliable preparation for the active rhodium(I) product. More detailed biological characterisation of the gold(I) compound found to be active in vitro would be valuable, including studies on its mechanism of action. The DNA-binding experiments applied to the rhodium(I) products could be improved further. Since the mechanism of action of these compounds is still not completely clear, future investigations might include apoptosis, cell cycle and various cell signalling studies.

Acknowledgements

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List of Abbreviations

- cisplatin – *cis*-diamminedichloroplatinum(II)
- dH_2O – de-ionised or distilled water
- DMEM – Dulbecco's modified Eagle's medium;
- DMSO – dimethyl sulfoxide;
- dppp –bis(diphenylphosphino)propane;
- EC_{50} – 50 % effective concentration;
- EDTA – ethylenediamine tetraacetic acid;
- FCS – foetal calf serum;
- G2/M – gap/growth 2 OR mitosis phase of cell cycle
- H (in the context of NMR), number of hydrogen atoms corresponding to a particular signal;
- ICP-MS – inductively coupled plasma-mass spectrometry;
- IR – Infrared (spectroscopy)
- KRB – Krebs-Ringer buffer;
- K-SFM – Keratinocyte serum-free medium;
- LB – Luria broth;
- MTT – (3-[4,5-dimethylthiazol-2-yl])-2,5-diphenyl tetrazolium bromide;
- NER – nucleotide excision repair;
- NMR – nuclear magnetic resonance;
- PBS – phosphate buffered saline;
- PIPES – piperazine-1,4-bis(2-ethanesulfonic acid);
- SDS – sodium dodecyl sulfate;
- TBE – Tris-borate EDTA;
- TE – Tris-EDTA;
- THF – tetrahydrofuran;
- TLC – thin-layer chromatography;
- Tris – 2-Amino-2-hydroxymethylpropane-1,3-diol;
- UV/vis – ultraviolet/visible light.

Chapter 1. Introduction

Chemistry and Biological activity of Cisplatin

Oesophageal cancer

Oesophageal cancer represents a significant health problem worldwide, especially so in the so-called 'cancer belt' of Asia (1) and parts of Africa, including South Africa. The squamous cell carcinoma subtype is most common in these developing countries (2,3), and is associated with various environmental factors, including smoking, drinking of alcohol and consumption of *Fusarium*-contaminated maize (4). Infection with various strains of the human papilloma virus may also be a risk factor (5). Because the disease is largely asymptomatic in its earlier stages, diagnosis often occurs only at an advanced stage, resulting in a 5-year mortality rate exceeding 90 % in South Africa (2,6).

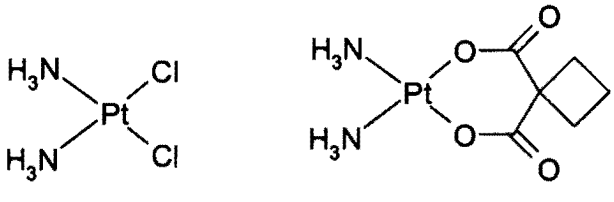
Surgery resection of the oesophagus remains the preferred front-line treatment against oesophageal cancer, depending on the stage at which the disease is diagnosed. Preoperative chemotherapy, alone or combined with radiation treatment, has been shown to yield modest advances in survival over surgery alone. Moreover, chemoradiation is often the sole treatment available for bulky or otherwise inoperable tumours. All of the standard chemotherapies rely on the use of the platinum-based drug cisplatin, often used in conjunction with other compounds such as 5-fluorouracil and epirubicin (7-9).

History of platinum based drugs

The antiproliferative activity of *cis*-diamminedichloroplatinum(II) (**1**), more commonly known as cisplatin, on *Escherichia coli* bacteria was discovered by

Rosenberg and colleagues in 1965. This was, in fact, a fortuitous accident, as these researchers had been intending to investigate the effect of an electric field on these bacteria – the compound was produced via a reaction between solutes in the nutrient solution and the platinum electrode (10). They subsequently demonstrated its potential as an antitumour agent (11). Cisplatin subsequently started being applied in clinical trials in the early 1970's and was approved for use by the US Food and Drug Administration (FDA) in 1978 (12). Its success has been most pronounced in the treatment of urogenital cancers - while testicular cancer, for instance, was once almost invariably fatal, cure rates may now exceed of 80 % (13).

Shortly after it was first introduced, the major limiting factor for cisplatin treatment was severe toxicity towards kidney and gastrointestinal tissue. This sparked an active research effort in developing an analogue of cisplatin that produced the same anti-tumour effect, but lacked this toxcity. The most notable outcome of this was carboplatin. This is characterised by a much slower pharmacokinetic profile than cisplatin (for reasons explained below), thus eliminating the short-term acute toxicity that produced the undesirable effects. On the other hand, this means that much higher doses are required to achieve similar effectiveness against cancer cells. Carboplatin was approved by the FDA for clinical use in 1989 (14). Together, cisplatin and carboplatin now constitute the most important metal-based anti-cancer pharmaceuticals in general use.



Cisplatin Carboplatin

Figure 1. Platinum-based drugs in common use.

Current research into platinum-based chemotherapies now centres on circumventing the innate or acquired resistance that some tumours display to cisplatin and carboplatin (more on this below). Oxaliplatin (Figure 2, A) is a more water-soluble variant of carboplatin, which retains activity against cancer cells with acquired resistance to cisplatin. Moreover, it has a broader spectrum of activity than cisplatin and carboplatin, proving valuable against colon cancers in combination therapy, for instance (15,16). Other developments include satraplatin (Figure 2, B), which is designed to an orally available drug with similar pharmacokinetic profile to carboplatin, and shows promise in this regard. The large methylpyridine ligand of picoplatin (Figure 2, C), is designed to provide steric hindrance around the metal centre, thereby preventing the thiol-coupling reactions that represent one of the main routes of cisplatin resistance. Indeed, this compound has shown promising activity against both cisplatin-sensitive and resistant cancers *in vivo* (17).

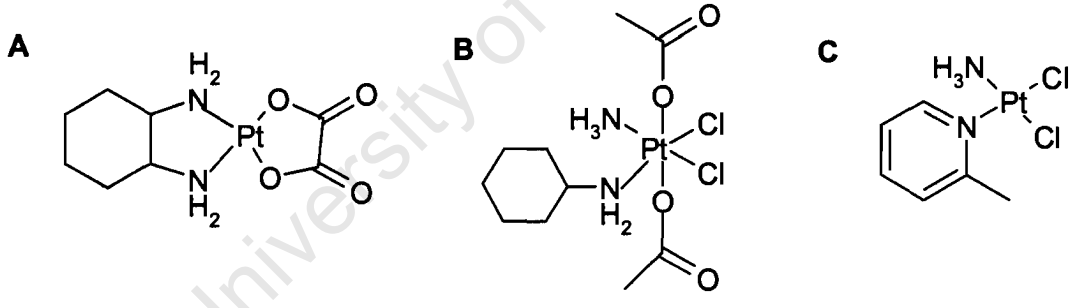


Figure 2. Specialised derivatives of cisplatin and carboplatin.

Chemistry and biochemistry of cisplatin

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Cisplatin had originally been prepared in 1845 by Michel Peyrone, after whom it was named Peyrone's chloride. Its structure was subsequently deduced by Alfred Werner, as part of his Nobel Prize-winning work in coordination chemistry

(18). The complex is prepared by a straightforward addition of ammonia to a tetrachloroplatinate salt (19). The cis- isomer is favoured over the trans-isomer because platinum(II) exhibits a marked trans effect, whereby a ligand is able to destabilise the ligand trans to itself. In this case, chloride produces a more pronounced trans effect than ammonia so, after the first ammonia molecule bonds to the metal centre, the second ammonia ligand is unlikely to displace the chloride trans to it, instead displacing one of the chlorides in the cis position. Selectivity can be improved further by replacing the chloride ligands with iodide, as these produce a stronger trans effect. The trans isomer can be produced, but only by complete replacement of all ligands by ammonia, then reintroduction of chloride (20).

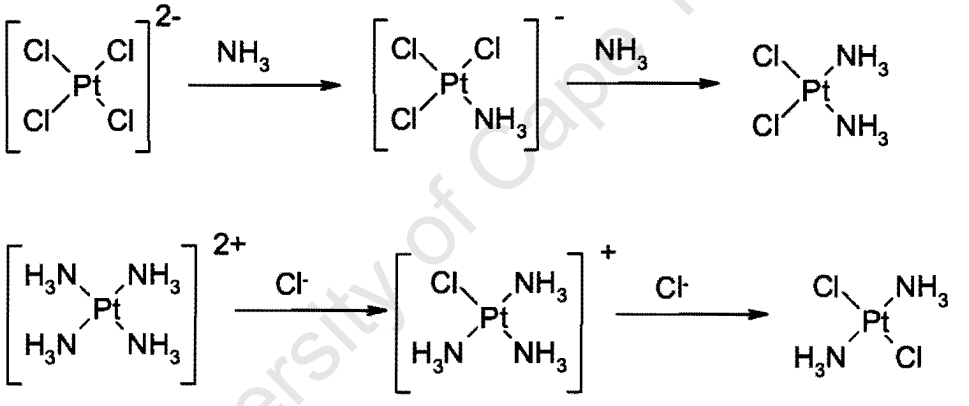


Figure 3. Preparation of cis- and transplatin

E. coli bacteria exposed to cisplatin grow in filamentous forms (21), and are more susceptible to lysis by bacteriophage λ (22). Both of these attributes are associated with DNA damage, implying that DNA is cisplatin's biological target. This hypothesis was later given support by studies showing that cisplatin prevented incorporation of radiolabelled nucleotides (23), suggesting inhibition of DNA synthesis. Furthermore, cisplatin co-isolates with DNA orders of magnitude more readily than it does with RNA and protein fractions from cisplatin-treated cells. This result is interpreted to indicate that cisplatin binds

with far greater affinity to DNA than to other biological molecules (24). Finally, using synchrotron radiation-induced X-ray emission (SRIXE), the concentration of an element like platinum can be mapped within individual cells, and is revealed to localise to the nucleus (25).

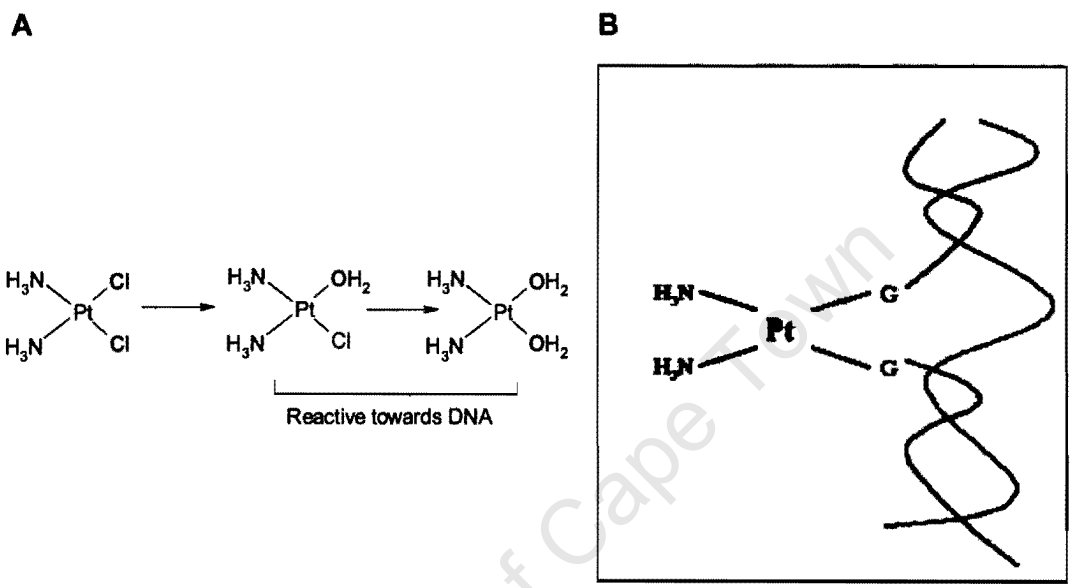


Figure 4. A: Hydrolysis of cisplatin. B: Depiction of cisplatin-DNA diadduct.

The available evidence suggests that cisplatin forms covalent adducts to purine bases on DNA, primarily guanine (26). If cisplatin is allowed to react with salmon sperm DNA, which is then thoroughly digested with nucleases, a mix of free nucleotides and nucleotide-cisplatin adducts is obtained. These products may be separated by chromatography and analysed by ^{1}H NMR. This experiment reveals that the majority of cisplatin is found in the form of intrastrand diadducts including adjacent guanine bases. Most of the remainder is found as intrastrand adenine-guanine diadducts (27) and intrastrand guanine diadducts where a single nucleotide separates the two guanine bases. Only a small fraction form interstrand guanine diadducts (28).

In aqueous solution, the rate-limiting step in cisplatin complexation to DNA is 'activation' via the hydrolysis of labile Pt-Cl bonds (Figure 4, A). The kinetics of hydration are strongly dependent on the concentration of chloride ion in solution. Thus, in plasma ($[Cl^-] \approx 100$ mM), cisplatin is relatively stable, whereas in the cytosol ($[Cl^-] \approx 20$ mM) the half-life for hydrolytic scission of a given bond is approximately 2 hours (29). The positively charged complex resulting from hydrolysis reacts rapidly with nucleophilic atoms on DNA, most commonly N7 on guanine. Although the reactive species of both cisplatin and carboplatin are identical, Pt-O bonds are not as labile as Pt-Cl bonds, meaning that carboplatin is hydrolysed far more slowly and possesses a different pharmacologic profile, as discussed above (30).

Effects on RNA transcription

DNA is involved in several metabolically important functions in the cell, and the disruption of any of these can potentially result in cell death. Because cisplatin forms adducts to DNA, a reasonable initial hypothesis is that it exerts its cytotoxic activity by inhibiting DNA replication. Indeed, both the bacterial Klenow fragment (31) and mammalian polymerase (32) halt progression at the site of these adducts. The formation of diadducts, as opposed to monoadducts, is necessary for this inhibition, and lesions on adjacent guanine bases are most effective in stalling polymerases (33). Cisplatin inhibits replication effectively at lower concentrations than the geometrical isomer transplatin, largely because it is stereochemically more able to form diadducts after initial monocomplexation (34).

If inhibition of replication was responsible for the cytotoxity of cisplatin adducts on DNA, we would expect cell cycle experiments to demonstrate that S-phase arrest is correlated with cell death, as this is the phase of the cell cycle in which DNA replication takes place. Sorenson and Eastman (35-37) have shown, as

expected, that S-phase arrest does occur and that the degree of inhibition of DNA synthesis is proportional to cisplatin concentration in various cell lines. However, using cell lines of differing sensitivity to cisplatin, they demonstrated that the characteristic feature of sensitive lines was the formation of G2/M arrest in response to cisplatin treatment. Cisplatin resistant lines generally underwent S-phase arrest of equal magnitude to sensitive lines, but then did not proceed to G2/M arrest. Therefore, although cisplatin brings about both G2/M and S-phase arrest, only the former is correlated with cisplatin cytotoxicity.

To account for this, the authors suggested that cisplatin toxicity is mediated by inhibition of RNA transcription, which would prevent the production of proteins necessary for passage into mitosis and so bring about G2/M arrest and eventual cell death. This hypothesis is supported by experiments demonstrating that RNA polymerases are unable to transcribe RNA from DNA reacted with cisplatin (38). To complicate matters, Cullinane *et al* (39) subsequently showed that RNA transcription from undamaged DNA could be inhibited simply by the introduction of a platinated plasmid into HeLa cells. This suggests that cisplatin-DNA adducts need not be present on the template DNA to inhibit RNA transcription, but merely be present in the cell. This suggests that these adducts may exert their effect by sequestering factors required for normal transcription to take place – termed the "hijack" theory. The crucial victims of this sequestration are believed to be protein factors common to both DNA repair mechanisms and the transcriptional machinery. For a comprehensive review of this theory, including proteins implicated to date, see Jamieson and Lippard (40).

Effects on telomeres

Because of their dependence on RNA primers, DNA polymerases are unable to replicate the ends of linear DNA molecules, leading to loss of information. To prevent loss of coding sequences, all mammalian chromosomes therefore

terminate in regions of long tandem repeats, of sequence $(TTAGGG)_n$. These regions, called telomeres, are degraded by approximately 50 bp with each cell division. The result of telomere depletion is that most cells will stop dividing and enter senescence after a certain number of divisions (41). Cancer cells, however, are able to replicate indefinitely. One factor accounting for this is that late-stage malignant cells abnormally express telomerase, an RNA-dependent DNA polymerase that is able to extend telomeres (42). Experiments in HeLa cells suggest that even a dose of cisplatin that is not immediately lethal can cause shortening of telomeres, potentially reversing cancer cells' abnormal replicative longevity. Indeed, cells treated at a low dose of cisplatin often do die after several divisions, although more work is necessary to link this conclusively to telomere depletion. The greater sensitivity of telomeres to cisplatin treatment can be accounted for by two factors. Firstly, telomeres have high guanine content and a highly complex secondary structure, rendering them vulnerable to disruption. Secondly, whereas coding sequence is normally replicated in early S-phase, telomeres are generally only replicated in late S-phase, thereby rendering them more vulnerable to the S-phase block characteristic of cisplatin (43).

Pro-apoptotic effects

Sorenson and Eastman (44-46) believe that the final mechanism of death for cisplatin-treated cells is apoptosis. This is confirmed by the accumulation of "sub-G1" cellular debris observable in cell cycle experiments performed on populations of treated cells. The DNA of affected cells develops double-stranded DNA nicks, producing a "ladder" in an agarose gel electrophoresis experiment that is characteristic of apoptosis. Finally, Lu and Cederbaum (47) have noted that cells treated with cisplatin for some hours undergo activation of caspase-3, -6, and -9, a biochemical step signalling that apoptosis is now irreversible within the cell.

Apoptosis, also termed "programmed cell death", occurs in response to severe cellular damage, especially to DNA. The process is initiated by detection of this damage by monitoring proteins, activating a signalling cascade. The end result of this cascade is the activation of proteases called caspases, which both directly degrade and indirectly bring about the degradation of cellular components, including the chromosomes and cell membrane (48). There is some evidence linking DNA repair machinery to induction of apoptosis. The primary means of repair of cisplatin lesions on DNA is the nucleotide excision repair (NER) system. While NER knockouts tend to exhibit increased cisplatin sensitivity (49), cell lines deficient in the mismatch repair system, another mechanism of DNA repair, tend to display cisplatin resistance (50). It is believed that the mismatch repair system, while recognising cisplatin lesions on DNA, is not able to repair them correctly. Instead, it attempts to 'repair' nucleotides on the strand complementary to that affected. As the lesion remains intact, the complementary nucleotides are continually recognised and continually being replaced. It is hypothesised that this 'futile cycle' of DNA repair creates a signal that enhances apoptosis (51,52). In light of this, it is interesting that testicular and ovarian tissue both tend strongly to express MSH2, an important component of the mismatch repair pathway, and that cancers deriving from these tissues are most effectively treated by cisplatin (53). Testicular tumours are also unusual among cancers in that they often have normal p53, a protein central in apoptotic signalling, particularly in the case of DNA damage. One might naturally speculate, therefore, that the presence of p53 renders these cancers vulnerable to induction of apoptosis by cisplatin (54).

Clinical limitations of cisplatin

A major problem associated with cisplatin is intrinsic or acquired resistance. This resistance may render a course of treatment ineffective, or allow it to be

effective for only a limited time. Another major concern linked to this is that of 'cross-resistance', whereby cells acquire resistance to both cisplatin and structural or functional analogues of the drug. It is hoped that these problems can be circumvented by the creation of novel cisplatin analogues (55).

Cells generally acquire resistance to cisplatin by one of three mechanisms. For a comprehensive review, see Kartalou and Essigmann (56). One mechanism of resistance is simply a reduction in the quantity of compound present in the cytoplasm, which is largely accomplished via decreased intake. The alterations undergone by cells to achieve this decreased intake remain incompletely understood, but several mechanisms are known. Cisplatin influx into cells is thought to be a process of passive diffusion, through the membrane or transmembrane protein channels or pores. Resistant cancer lines may have mutations in these channels that prevent efficient passage of cisplatin. Channels of this sort are often 'gated' by the electrical potential across the membrane, accounting for the observation that some cisplatin-resistant cell lines maintain hyperpolarisation across their membrane (57).

In recent years, particular attention has been paid to the copper homeostasis system (58). In particular, it is now believed that one of the major influx routes for cisplatin is copper transporter-1 (CTR1), a transmembrane pore protein. $Ctr1^{-/-}$ mammalian cells are found to accumulate only around 35 % as much platinum as wild type cells when exposed to clinically relevant concentrations of cisplatin (59). Furthermore, exposure to potentially cytotoxic levels of either copper or cisplatin brings about rapid downregulation of CTR1 protein, via internalisation and proteasome degradation (60). ATP-dependent efflux proteins associated with copper transport, including ATP7A and ATP7B, are also associated with cisplatin resistance. Increased expression of these proteins is linked to decreased platinum concentration in cells, and to reduced cell death (61).

Cisplatin may effectively be detoxified by coupling to cellular thiol peptides, the best understood of which is glutathione. Attachment to glutathione precludes the formation of adducts to DNA, diminishing toxicity. It has been demonstrated (62) that some cisplatin-resistant cancer cell lines overexpress glutathione. In addition to the initial detoxification, once cisplatin is conjugated to glutathione, the entire assemblage can be exported from the cell via an ATP-dependent pump that recognises glutathione (63).

Because cisplatin exerts its cytotoxic activity by inflicting lesions on DNA, cells are able to eliminate this toxicity by increasing their repair of these lesions. Thus cisplatin-resistant cell lines have been demonstrated to undergo more efficient DNA repair (64). The most important upregulated pathway is that of nucleotide excision repair (NER), whereby several bases on either side of the lesion are removed and replaced (65). Interestingly, constitutive deficiency in this pathway is now believed to be one of the major reasons why testicular cancer is so responsive to cisplatin therapy (66). As mentioned above, the mismatch repair pathway is unable to repair cisplatin lesions, but may contribute to their toxicity. Cancer cells therefore tend to lose this pathway as they develop cisplatin resistance (67).

A major problem with cisplatin is severe toxicity, particularly nephrotoxicity (55) caused by the apoptotic death of healthy cells (68,69). Although nephrotoxicity can be controlled by intravenous hydration and administration of diuretics (70), this still tends to be the limiting factor in successful treatment of patients (55). Cisplatin's effects on renal tissue are therefore the best studied. Apoptosis appears to be promoted in the renal tissue via multiple routes, including inhibition of protein synthesis (71), as well as damage to the mitochondria (72,73) and the lysosomes (74). It has also been demonstrated that, in rat renal tissue, cisplatin treatment causes activation of the tumour necrosis factor family of receptors, leading to apoptotic cell death (75).

The worldwide success of cisplatin and, conversely, some of the problems associated with cisplatin treatment, have spurred a very active worldwide research effort into the biological properties of precious metals. Various other platinum compounds have been developed as potential therapeutics. For a review of platinum compounds designed to circumvent resistance, see Kelland et al (76). While problems with toxicity have been partially alleviated using analogues such as carboplatin (77), these variants often display cross-resistance with cisplatin. In recent years, interest has grown in compounds based on other metal centres, as the opportunities for cross-resistance are more limited here. However, many of these compounds share sufficient chemical similarities with platinum(II) to allow for biological activity. Complexes of Au (78), Ru (79), Pd, Ir (80) and Rh (81) have been tested, although very few have reached clinical trials and none have as yet been certified as drugs (80).

Chemistry and Biological activity of Rhodium(I)

Introduction to Rh(I) chemistry

The most easily attainable oxidation state of rhodium is rhodium(III). However, the +1 oxidation state has garnered interest in recent years, largely due to its catalytic properties. The extremely successful Wilkinson's catalyst is centred on a rhodium(I) centre. Rhodium(I), like platinum(II), has a d^8 electronic configuration, and its complexes are generally four-coordinate and square planar. All known isolable complexes of rhodium(I) contain at least one π-acceptor ligand, which stabilizes and prevents oxidation of the metal centre. The most common ligands seen in catalysts are tertiary phosphines. The relative bulk of these ligands tends to result in coordinative unsaturation via ligand dissociation, enhancing catalytic activity (82).

Chatt and Venanzi (83,84) produced the first coordination compound of rhodium(I) with a diene, 1,5-cyclooactadiene, to produce a chloro-bridged dimer. In the same work, they describe bridge-splitting reactions of this dimer with phosphines and amines, including piperidine and 2,2'-bipyridine. Partenheimer and Hoy (85) describe the first such reaction with pyridine as a ligand. In the same year, Pannetier and coworkers (86,87) prepared various chloro(1,5-cyclooctadiene)(amine)rhodium(I) compounds, where the amine included pyridine, vinylpyridine and methylpyridines. They demonstrated that the cyclooctadine ligand may be displaced by carbon monoxide under atmospheric pressure, and carried out infrared studies.

Recently, Rajput et al (88-90) report the preparation of a series of complexes of the type (1,5-cyclooctadiene)(chloro)(L)rhodium(I) with L a substituted pyridine. They place particular emphasis on ferrocenyl, phenyl and other aromatic systems as substituents on the pyridine ring. These investigators have demonstrated that these sorts of compounds display interesting voltammetric properties, due to their conjugated systems of double bonds.

Rhodium(I) complexes in cancer therapy

Most of the recent interest in rhodium as a potential anti-cancer compound has centred on the +2 oxidation state, usually found in dimeric form and in conjunction with bidendate carboxylate ligands (Figure 5). These compounds are found to be cytotoxic *in vitro* and to be effective at inhibiting tumour growth in a nude mouse model . Moreover, the biological effectiveness is found to correlate positively with the length of the carboxylate aliphatic chain, which is thought to be due to the increased ability of more hydrophobic compounds to cross the cell membrane (91,92). These complexes have been found to interact with human serum albumen (93) and, more importantly, with DNA (94,95).

presence or absence of crucial DNA repair enzyme made no difference to the effectiveness of the treatment.

Giraldi et al (99) went on to show that compounds of this type are also useful in a mammalian system (the compounds referred to by letters **A-F**, hereafter signify those so denoted in Figure 6). These workers demonstrated that **D** and **F** inhibit tumour growth in murine models of Ehrlich ascites carcinoma. *In vitro*, the compounds markedly inhibited protein synthesis after 4 hours, with far lesser effect on RNA and DNA synthesis. This contrasts with the inhibition of DNA synthesis observed for compound **A** and for cisplatin, suggesting a different site of action. Under these conditions, even the most active rhodium compounds tested displayed lower activity than Pt(II) complexes. The authors attributed this to the high lability of the Rh-Cl bond compared to the Pt-Cl bond and to the potential for Rh(I) to be oxidised to Rh(III) under cellular conditions.

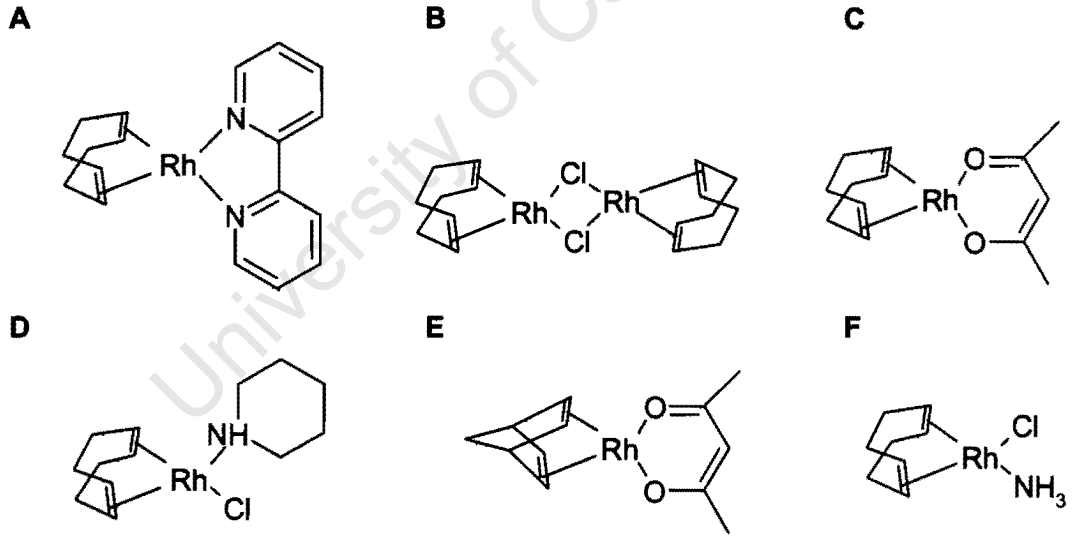


Figure 6. Some Rh(I) complexes previously shown to possess some activity against cancers

Giraldi *et al* (100,101) report that compound **C** possesses antitumour activity that compares favourably to that of cisplatin. This complex attained complete cures in a murine model of Ehrlich ascites carcinoma at comfortably sublethal doses and inhibited metastasis in a model of Lewis lung carcinoma (102). Moreover, they report that the rhodium compound was less toxic than cisplatin, as measured by lesser reduction of body weight in the treated mice and less histologically apparent damage to the kidney, intestinal mucosa and spleen. Interestingly, they explain this selectivity in the same way they account for the lack of efficacy in **D** and **F**, in terms of the instability of these compounds to oxidation. Because the cytoplasm of a cancer cell is more reducing than that of a normal cell, they speculate that the compounds remain in their cytotoxic form for longer in these malignant cells. By monomer incorporation experiments, **C** can be shown to impact primarily upon RNA synthesis (103,104). **E** and, to a lesser extent, **B** are also reported to display some activity, but less than that of **C**, suggesting that both the carboxylate anion and the form of the diene ligand are important for biological activity. This may be rationalised in terms of stability effects, as the cyclooactadiene bidentate ligand (**A-C**, **F**), as opposed to the norbornadiene ligand of **E**, stabilise the +1 oxidation state of the Rh atom. Similarly, Rh-O bonds are generally more stable than Rh-Cl bonds, whereas bidentate ligands tend to be kinetically inert (105,106).

The same group of investigators have also prepared a related series of compounds with pyridine-based ligands (Figure 7) (107,108). These were found to be effective against a mouse model of MCa murine mammary carcinoma, and to inhibit metastasis of Lewis lung carcinoma. On the basis of histological observations of other tissues, the authors conclude that the reduction in metastasis is attributable to alterations induced in the primary tumour, rather than the effect of the compounds toxicity on secondary tumours.

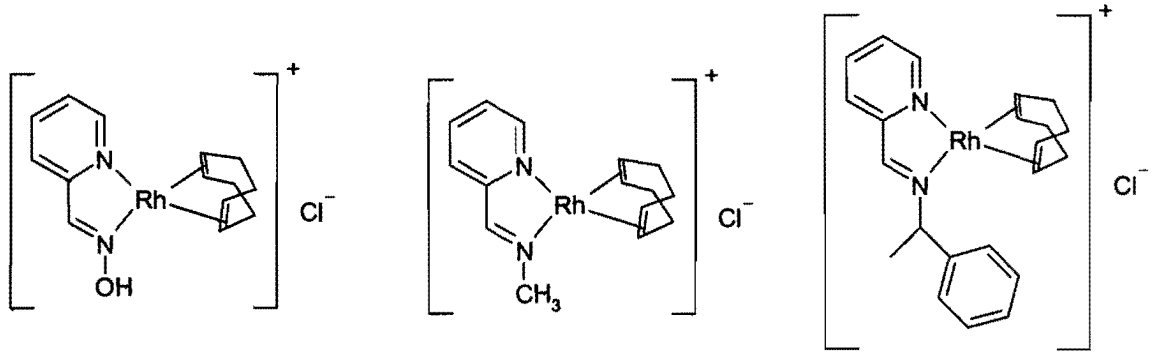


Figure 7. Rhodium(I) compounds with pyridine-based ligands and anticancer activity.

Rajput et al (88), have recently found several neutral and charged complexes of 1,5-cyclooctadiene stabilised rhodium(I) compounds with pyridine-based ligands to have *in vitro* activity approaching that of cisplatin. The most effective of these are shown in Figure 8. In this work, it was decided to explore the more compact neutral complexes in more detail.

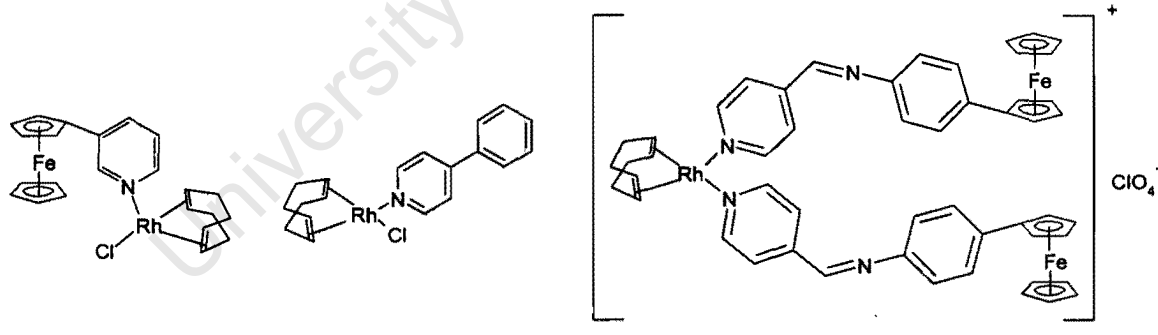


Figure 8. Biologically active rhodium complexes prepared by Rajput *et al* (89).

Chemistry and Biological Activity of Gold(I)

Introduction to Gold(I) Chemistry

Gold(0) has the electronic configuration $[Xe]4f^{14}5d^{10}6s^{1}$. The nuclear charge of gold is very large, exerting a strong force, and thus a powerful acceleration on the electronic orbitals. The result of this is relativistic alterations to the expected energy levels of these orbitals. In particular, the 5d orbital is destabilised (enlarged), whereas the 6s and (to a lesser extent) 6p orbitals are stabilised/contracted. This effect is responsible for the resistance of metallic gold to initial oxidation, as compared the other elements in the same row of the periodic table, copper and silver. However, once oxidised, gold is able to enter much higher oxidation states than these metals, due to the relatively small gap between the 5d and 6s orbitals (109). This accounts for the tendency of Au(I), in aqueous solution, to disproportionate according to the following equation (110):

$$3Au(I) \rightarrow 2Au + Au(III)$$

Gold(I) has the closed shell electronic configuration $[Xe]4f^{14}5d^{10}$, isoelectronic to that of Zinc(II). This species is most commonly found complexed to two ligands, with linear stereochemistry (110). However, trigonal planar three-coordination, and tetrahedral four-coordination are also known (111). In contrast, in copper and silver, the +1 oxidation state tends to form four-coordinate cluster compounds. This is because relativistic contraction of the 6s orbital causes the 6s-6p to be considerably greater in gold than the equivalent gap in Cu and Ag. This energy gap favours sp hybridisation, and therefore two-coordinate linear stereochemistry (112).

Gold(I) is a relatively "soft" metal ion, as we would predict from its large size and low oxidation state. It therefore has greater affinity for, and is stabilised by, large

anions such as I^- and CN^- than for the lighter halides (109). However, there is also a pattern of anti-symbiosis, whereby a soft ligand can destabilize the bond of another soft ligand trans to itself. Thus the ligand $P(OPh)_3$ tends to favour thiocyanate bonding to gold(I) via N, rather than through the softer S. If we ignore anti-symbiosis, the relative affinities of gold(I) towards various neutral ligands are as follows (110):

$Ph_3PO < Me_2S <$ pyridine $< AsPh_3 < NH_3 << PPh_3 \sim$ MeNC $< PMePh_2 < PMe_2Ph$

for biologically important ligands (113):

$COO^- << Cl^- <$ His(=N-) ~ Met-S-$CH_3 << CN^- \sim PR_3 << CysS^-$

and the halides (113):

$F^- << Cl^- < Br^- < I^-$

We note that the most favoured neutral ligands are phosphines, accounting for the general preference for these ligands seen in the literature. We can also see that that pyridine is not a particularly favoured ligand.

Linear complexes of gold(I) are susceptible to a "*trans* influence", whereby a ligand is able to destabilize the bond of the metal centre to the substituent *trans* to itself. The strength of this effect is thought to increase with the σ-donor ability of the ligand. As successively stronger σ-donors are used, the bond to the *trans* ligand assumes a successively more diffuse π-bond character, and the measured bond length between it and the metal increases (110). Jones and Williams (114) have carried out measurements of this *trans* effect in the [ClAuL] system, where L is varied systematically. The results of this demonstrate that

pyridine, in particular, is a weak σ-donor and strong π-donor, as one might expect. Thus it exerts a weak *trans* effect relative to ligands such as phosphines.

There have been numerous published examples of Au-C σ-bonds. These are most commonly prepared by reaction of an organolithium or Grignard reagent with gold(I) halide. This gives, by displacement of the halide, [RAuL], where R is the organic group and L is usually a tertiary phosphine. R groups include alkyls, phenyls, pentahalophenyls, ferrocenyl and η^1-bonded cyclopentadienyl (110). The pentafluorophenyl anion is commonly used as a counter-ion in gold(I) compounds.

The first preparation of (pyridine)gold(I) chloride is recorded from 1930 (115), and similar products can now be synthesised relatively routinely (116). Barranco *et al* have prepared complexes of the type [(3-ferrocenylpyridine) Au(I) X], where X is Cl or C_6F_5, via displacement of tetrahydrothiophene by the aromatic nitrogen.(Figure 9, A) These are found to be air- and moisture-stable red solids that are not conductive in acetone solution (117). The same investigators has also prepared similar ferrocene-containing complexes with the ferrocene moiety attached to a pyrazole (118) or a pyridine group (119) via a linker. (Figure 9, B). In a similar vein, Aguado et al have prepared an unusual η^3-M_3 complex, with each nitrogen of a ferrocene-linked terpyridine moiety coordinating Au(I)(C_6F_5). (Figure 9, C) As there are multiple gold atoms in this complex, aurophilic interactions contribute to its stability (120).

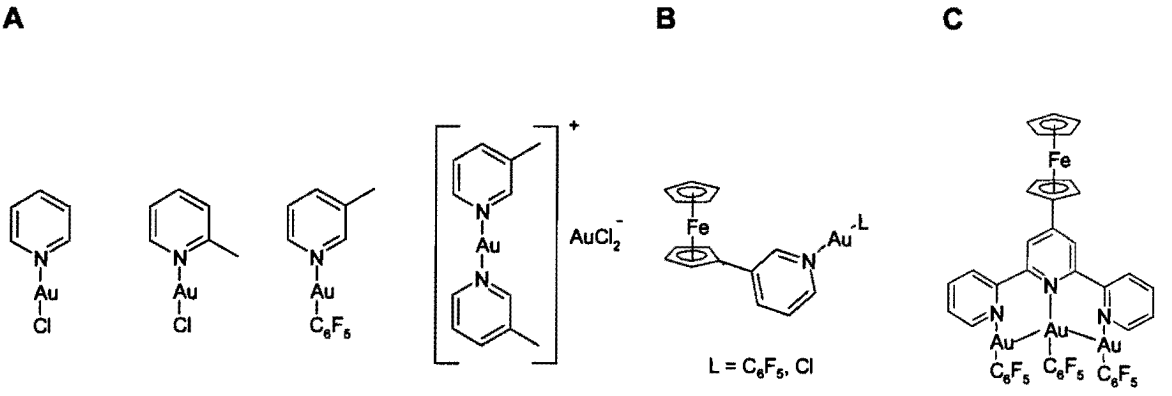


Figure 9. Reported gold(I) compounds with substituted pyridine ligands

Medicinal use of gold(I)

The most conspicuous medicinal application of gold at present is in antiarthritic drugs. These primarily take the form of gold(I) thiolates and phosphines (see Figure 10). The first of these was aurothioglucose, followed shortly afterwards by other thiol-based drugs. All of these must be injected for clinical effectiveness. The only orally available drug in this class is auranofin, which has both a thiosugar and a phosphine moiety. The mechanism by which these compounds alleviate the disease condition remains uncertain, but anti-immune activity is thought to play a prominent role (113,121). Following on the success of auranofin, the literature on gold(I) compounds in treating cancer has centred on phosphine ligands. Auranofin itself has proved cytotoxic against HeLa cells *in vitro* and P388 leukaemia cells *in vivo* (113,122).

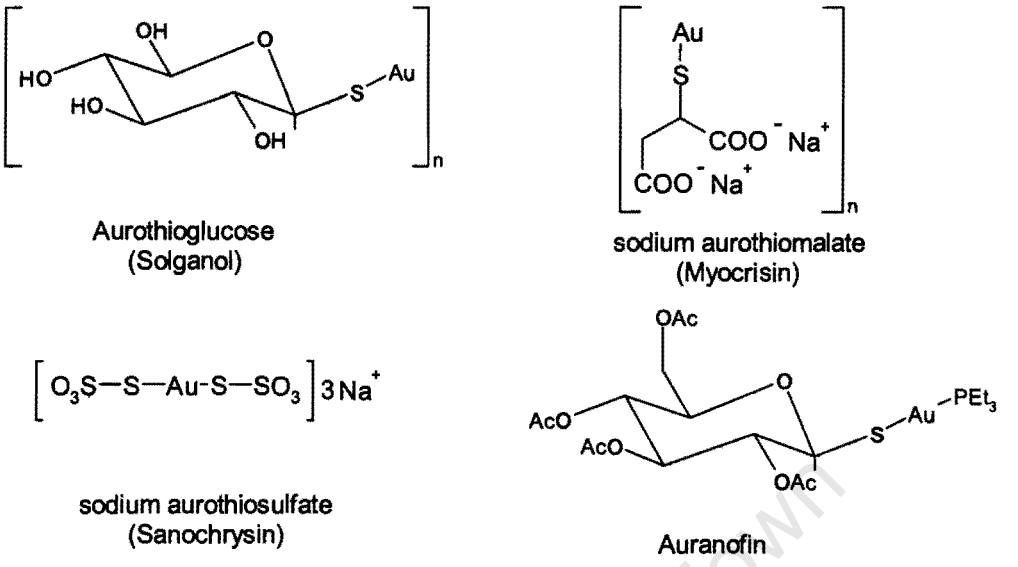


Figure 10. Gold(I) complexes used in anti-arthritis therapy

Two distinct classes of phosphine-gold(I) anticancer compounds are presently being investigated for anticancer activity, and have recently been reviewed by Bernand and Berners-Price (123). The first class, neutral linear two coordinate Au(I) complexes, (see Figure 11, A) includes auranofin (124), $(Et_3P)AuCl$ and many other compounds (125,126). These are thought to exert activity by interfering with an important anti-oxidant system in mitochondria. The proper functioning of many thiol-containing enzymes is compromised by oxidation with hydrogen peroxide formed as a by-product of respiration in the mitochondrion. The peptide thioredoxin itself is oxidized as it restores these oxidized proteins. This "redox buffer" is maintained by the enzyme thioredoxin reductase, which reduces thioredoxin at the expense of oxidation of NADPH to $NADP^+ + H^+$. Auranofin and related compounds have been shown to inhibit the action of thioredoxin reductase (127). This prevents the repair of mitochondrial enzymes damaged by oxidation. More importantly, an excess of the oxidized form of thioredoxin is a crucial signal promoting mitochondrion-mediated apoptosis (128).

The second class of phosphine-gold(I) anticancer compounds includes complexes with bis-coordinate phosphine ligands. Figure 11, B) These generally have tetrahedral geometry and are cationic. These complexes appear to induce membrane permeability in the inner mitochondrial membrane, which is a key step in apoptosis. However, in variants with high lipophilicity, this membrane permeabilisation appears to occur in all cell types, instead of preferentially in cancer cells. This effect may be responsible for the severe *in vivo* hepatotoxicity that has resulted in several promising compounds being abandoned in pre-clinical trials. The authors of the review suggest that more hydrophilic variants may be more selective for cancer cells, resulting in more acceptable toxicity. In this vein, Caruso *et al* (129) report a hydrophilic neutral phosphine-gold(I) compound with good activity against a panel of cancer cell lines. Pillarsetty and co-workers report similar results for an ionic complex, and link cell death to G1 cell cycle arrest (130).

Amine-gold(I) complexes are relatively under-represented in the medicinal chemistry, most likely because of the relatively stability of these complexes compared to the phosphine analogues. However, some experiments on (pyridine)gold(I) chloride are reported by Mirabelli *et al* (131). The authors demonstrate that this compound is able to interact with DNA in a cell-free *in vitro* system, is able to produce singe-strand breaks in the DNA molecule, as assayed by the interconversion of supercoiled and open circular plasmid DNA.

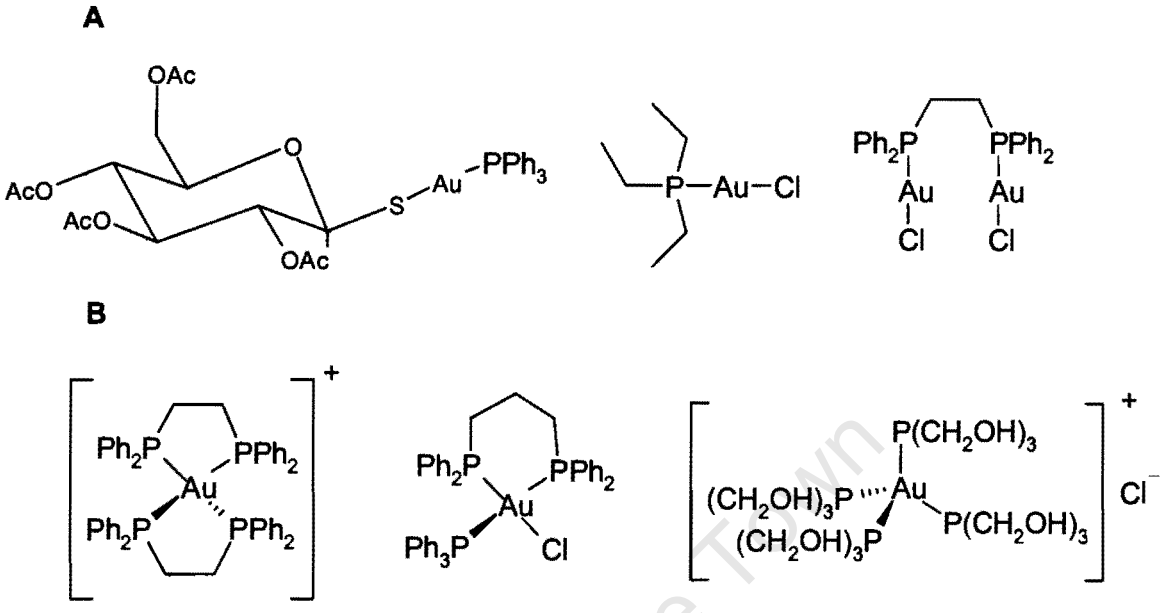


Figure 11. Some examples of gold(I) phosphine compounds with anticancer activity

Chemistry and Biological Activity of Gold(III)

Introduction to Gold(III) Chemistry

The +3 oxidation state is dominant in gold chemistry, although very rare in copper and silver. This is accounted for by the relativistic destabilisation of the 5d orbitals, as described above. Gold(III) has electronic configuration $[Xe]4f^{14}5d^{8}$, with all known complexes existing in the low-spin state. In addition to being isoelectronic to Pt(II), nearly all gold(III) complexes also have four-coordinate square-planar stereochemistry, although there have been reports of five-coordinate square pyramidal and six-coordinate trigonal bipyramidal

geometry. The former may be invoked as an intermediate state for ligand substitution in square planar complexes (110). Interestingly, as is often seen in square-planar geometry, gold(III) complexes exhibit a marked trans-effect. This means strong σ-donor ligands are able to labilise the bond trans to themselves (110,132).

The oxidation of metallic gold to gold(III) is associated with a large redox potential, and the gold(III) species is only stable in an aqueous system at low pH. This explains why a strongly oxidising acid, aqua regia, is required to oxidize gold. The presence of ligands other than water reduces this redox potential significantly. However, even with a hard chloride ligand, this potential is still of the order of 1.0 V. Gold(III), because of its relatively high charge, is a "harder" Lewis acid than gold(I). As such, it has a higher affinity for nitrogen donors. Complexation to such ligands can usually be achieved by simple displacement of chloride from the tetrachloroaurate anion (110).

Pyridinetrichlorogold(III) was first prepared in 1903 by Renz and, independently, by François (133,134). The latter also prepared $[(C_5H_5N)_2AuBr_2]Br$, by recrystallisation of pyridinetribromogold(III) from pyridine. This compound is soluble in ethanol, acetone and chloroform, but sparingly soluble in ether and insoluble in water (135). Catallini *et al* have carried out a series of kinetic studies on pyridinetrichlorogold(III) and related complexes. They have found that the pyridine ligand of a gold(III) complex is considerably more susceptible to substitution by anions than the analogous ligand in a Pt(II) system. This may be accounted for by the greater charge of the gold(III) centre, which allows it to form five-coordinate transition states necessary for associative kinetics. Because this transition state has a significant ionic character, the uncharged amine is favoured as the leaving group instead of the chloride ions (136). They have also determined that more basic ligands have more rapid kinetics of complex formation with gold(III) (137). Similar effects are observed for the micropolarizability ("softness") and the π-donating ability of the incoming ligand

(138). By the same token, it is observed that rate of displacement of pyridine-based ligands also varies positively with basicity (139-141). These effects are also observed with platinum(II), but make far less difference to the reaction kinetics. These authors note, however, that steric hindrance plays a far more significant role in gold(III) compared to platinum(II), particularly in the case of relatively bulky thioether ligands (142).

Aurocycle complexes including pyridine

Metallocycles with pyridine N-donation have been prepared extensively, often with a view to exploring their photochemical properties (143). The most common approach to this is either via aliphatic amines that are substituents of the pyridyl moiety (144), or via bipyridal ligands (137). While bidentate N,N-donor ligands are more frequently seen in the literature, varieties of C,N-donor aurocycles have also been prepared. Vicente *et al* prepared 2-[(dimethylamino)methyl]phenylgold(III) in 1984 (145), while Parish and coworkers have prepared a variety of analogues (146).

Constable and coworkers have used several analogues of 2,2'-bipyridine in the preparation of metallocycles, with a variety of metal centres (see (147-149)). In a particularly interesting work, they have demonstrated "roll-over" cycloauration of 2-phenylpyridine (150). Parish *et al* (151) have shown that, while these complexes can be prepared by direct reaction of ligand with the tetrachloroaurate anion, transmetallation of [{Hg(2-phenylpyridine)Cl}$_n$] with tetrachloroaurate gives greater yields. In this work, they also confirm that a soft donor atom will tend to displace chloride *trans* to the heteroaromatic nitrogen in these complexes, as expected by the *trans*-effect. Che and coworkers have extended this transmetallation methodology to form C,C,N-donor chelates of gold with 2,6-diphenylpyridine (152). Fuchita *et al* have prepared analogous six-member C,N-donor aurocycles (153,154) as have Nonoyama *et al* (155,156).

Fuchita and coworkers have also prepared several imidazole (157) and thiophene (158) analogues of these complexes. Various poly-N-donor terpyridine complexes have also been described (159).

Medicinal uses of gold(III)

Because of their similarity to platinum(II) complexes, compounds of gold(III) might be expected to possess utility as anti-cancer drugs. Nevertheless, interest in this field has grown only recently. A review from 1994 (113) refers to gold(III) primarily in the context of toxic side-effects of gold(I) drug compounds! A further problem is that many gold(III) complexes are unstable under biological conditions, mainly due to reduction to metallic gold (160).

Parish and colleagues have prepared various compounds of gold(III) with a view to biological testing (161). In their more recent publications, these workers recommend the use of soft carbon-donor ligands, especially aromatic groups, in order to stabilize the metal centre against reduction. They report that one of these compounds (**Figure 12**, A) displays good cytotoxic activity against various cell lines in vitro, but that studies on xenograft mouse models are disappointing, possibly due to the low aqueous solubility of the compound (162). They note significantly improved *in vivo* activity with a more water soluble analogue (**Figure 12**, B). Using a gel electrophoresis mobility study, this compound was demonstrated to interact with DNA, but only minor effects were noted on the cell cycle (163).

Henderson *et al* have prepared various 5- and 6-member aurocycles, using chloro, acetate and thiosalicylate anions (**Figure 12**, C), and observed modest anti-cancer activity in some of the resulting compounds (164). They have also prepared complexes with catecholates (165) and imidates (166). Several of these derivatives are demonstrated to be active against the P388 murine

leukaemia cell line, although poor solubility is a problem with some of these compounds.

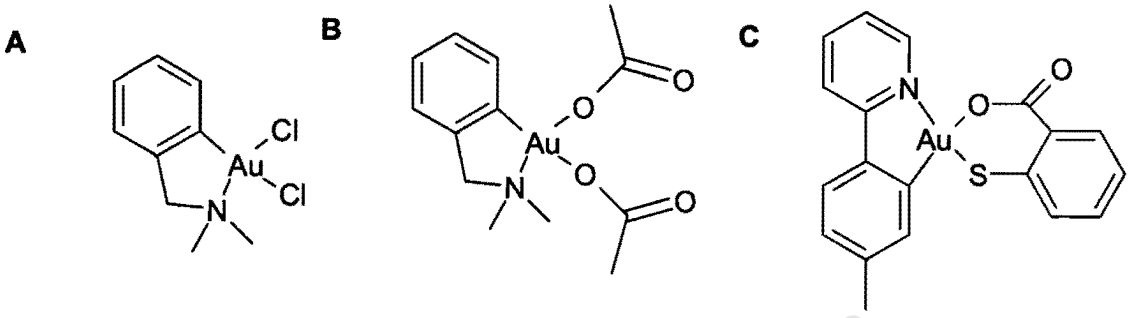


Figure 12. Aurocycle complexes of gold(III) with antitumour activity.

Messori and coworkers report the preparation of (2-pyridylmethanol)trichlorogold(III) and the N,O-donor aurocycle of this compound (Figure 13, A). They note that these compounds have poor solubility in water, potentially complicating their utility as biological agents. However, they are found to bind strongly to protein and DNA, and are reasonably cytotoxic towards cancer cells *in vitro*, although not as powerful as cisplatin (167). The same group have also reported that chloro glycylhistidinate gold(III) is considerably more cytotoxic than its analogues containing platinum(II), palladium(II), zinc(II) or cobalt(II), suggesting that the gold(III) centre is necessary for biological activity (168). They also note some cytotoxicity for bi- and tridentate nitrogen-donor ligands of gold(III) (169), although in this case the biological activity of the ligands in isolation was found to exceed that of the complex. However, Shi *et al* report that a 1,4,7-triazacyclononane complex of gold(III) shows cytotoxic activity comparable to cisplatin *in vitro* (Figure 13, B) (170) N,N- and C,N,N-donor bipyridine based complexes gave more promising activity (Figure 13, C) (171,172). Most of these complexes, though not the bipyridine derivatives, were found to bind DNA *in vitro*.

Messori *et al* have recently focused on these terdentate C,N,N-donor complexes. In particular, they have varied the anion at the fourth coordinating position, with a view to improving solubility and stability in aqueous medium. In general, these compounds were found to be stable for several hours under physiological conditions, and to exhibit reasonable toxicity against some cancer cell lines. As before the most active compounds were found to possess acetate cations (Figure 13, D) (173). These compounds were found to exert cytotoxic effect via apoptosis, as verified by cell cycle and TUNEL assays (174).

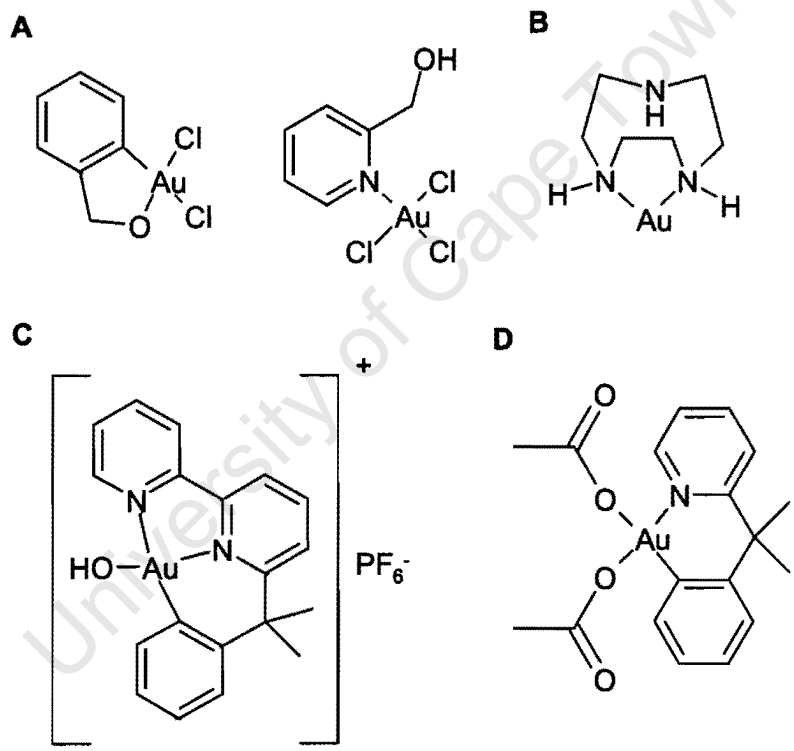


Figure 13. Some cytotoxic complexes of gold(III)

Sun and coworkers have recently done some interesting work on gold(III) porphyrins. Some tetraarylporphyrin complexes were shown to have significantly greater activity against cancer cells than cisplatin, and to exert this activity through an apoptotic pathway (175). The work of Gibbs, Pasternack and

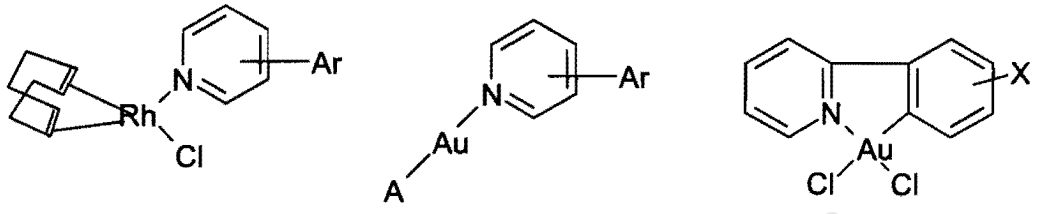
coworkers may shed some light on these results. These latter investigators have demonstrated that the gold(III)tetrakis(4-N-methylpyridyl)porphine is able efficiently to intercalate into DNA *in vitro*, and localizes to the nucleus in living cells (176).

Objectives of this work

The primary aim of this project is to identify precious metal compounds with promising anticancer activity, focussing on three metal centres – gold(I), gold(III) and rhodium(I). Gold(III) and rhodium(I) are chemically similar to platinum(II) introducing the possibility that they may exert comparable biological activity, while avoiding some of the problems associated with cisplatin treatment. Additionally, unlike say palladium(II) (177), the biological properties of these species have not yet been thoroughly investigated, meaning that there is scope for novel discoveries. Gold(I) is already known to have biological activity (113), although by a different route to cisplatin (123), and may therefore serve as a useful contrast to the other metal centres.

In this work, the decision was made to focus primarily on pyridine-based ligands, as opposed to phosphines, thiols, or any of the many organic ligands available. There were several reasons for this. Firstly, this work largely follows on from the results of Rajput *et al* (88-90), wherein certain pyridine-based complexes of rhodium(I) were found to be biologically active against oesophageal cancer cells *in vitro*. Secondly, although complexes of platinum(II) containing pyridine ligands are, due to their close similarity to cisplatin, fairly well-represented in the literature (178) pyridine complexes with other metal centres are rare. This is even the case where (as in this study) many of these ligands are quite readily available commercially or by simple synthesis. Therefore, from a chemical point of view, there is a certain interest in preparing and characterising such novel compounds. Thirdly, and especially in contrast to the phosphine ligands (179),

pyridine-based ligands are less intrinsically toxic, meaning they are easier to work with in a laboratory setting. This also means that any observed toxicity of the resulting complex may readily be attributed primarily to the metal centre, rather than the ligand. The complexes to be studied are depicted in Figure 14.



Ar = aromatic group; A = anion; X = substituent

Figure 14. Generalised structure diagrams for the complexes studied in this work.

Successfully prepared complexes are to be characterised by standard methods, including nuclear magnetic resonance (NMR), elemental analysis, melting point analysis (m.p.) and UV-Visible spectroscopy. Biological activity is to be investigated via several methods. The most basic criterion against which to judge the value of potential anticancer drugs is cytotoxic activity with respect to cancer cells. This can be evaluated against oesophageal cancers cells grown *in vitro*, using several well-understood methods.

Since there is little value in compounds that are simply indiscriminately toxic towards human cells, more insight must be gained into the mechanism of action of these compounds. Firstly, the cytotoxic activity of test compounds on cancer-derived versus relatively normal cell lines can be compared. This gives some idea of the therapeutic index, or selectivity, of potential drugs. There are several techniques that may be used further to probe the mechanism of cytotoxicity. Since cisplatin is known to exert activity via covalent attachment to DNA, several DNA-binding assays may be performed. These generally detect topological

changes induced in the DNA molecule by the attachment of treatment compounds, using measures such as gel migration and circular dichroism.

In summary, the complexes to be prepared include:

- gold(I);
- gold(III); and
- rhodium(I)

complexes with pyridine based ligands.

These compounds are then to be characterised via:

- NMR;
- Elemental analysis;
- UV-Vis spectroscopy;
- Cytotoxicity studies;
- and DNA-binding assays.

Chapter 2. Synthesis and characterisation of ligands and complexes

Results and Discussion

Preparation of ligands

Preparation of ferrocenylpyridine ligands

The preparation of these ligands was attempted, at least in the first instance, by reaction of the diazonium salt of the appropriate aminopyridine with ferrocene. Unfortunately, however, it soon emerged that this technique was only appropriate for the 3-isomer. This is a consequence of the relative lability of different isomers of the diazonium reagent. The diazonium salt of 3-aminopyridine reacts via a relatively slow S_N^2 pathway. In contrast, the corresponding diazonium salts of 2- and 4-aminopyridine are able to undergo rapid S_N^1 loss of nitrogen gas. (Figure 15) This is possible because the loss of the diazonium moiety results in a carbocation that is stabilised by resonance donation of electrons from the nitrogen, whereas the equivalent 3-isomer would not be so stabilised. Therefore, the 2- and 4-isomers are able to react rapidly via the S_N^1 pathway, often taking up chloride ions present in the hydrochloric acid solution in which diazonium salts are usually prepared (180).

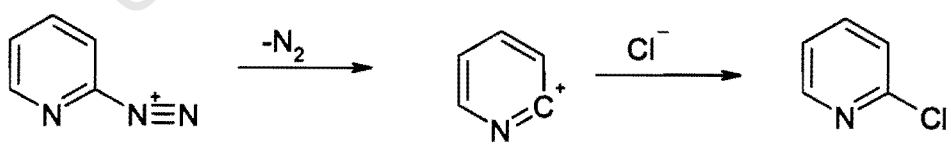


Figure 15. Spontaneous decomposition of the diazonium salt of 2-aminopyridine, and reaction with chloride present in hydrochloric acid solution.

The reaction of ferrocene with the diazonium salt of 3-aminopyridine was attempted via a phase-transfer methodology (181). However, it was found that several side-products were formed and that yields were generally low, resulting in a large excess of starting material in the reaction mixture. Because of this, pure product could not be readily obtained, even by column chromatography. Therefore, a more "old-fashioned" technique (182), exploiting the mutual solubility of the diazonium salt and ferrocene in glacial acetic acid, was employed. (Figure 16) After extensive diethyl ether extraction of the resulting product mixture, more than 2 g of 3-ferrocenylpyridine was obtained.

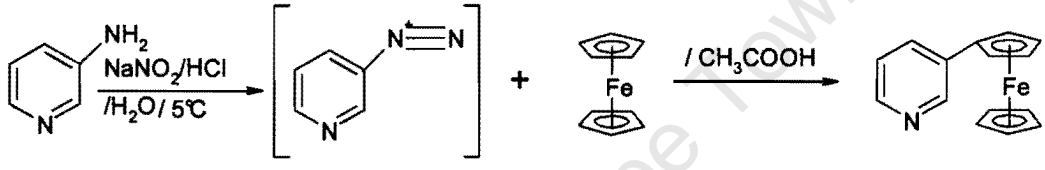


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Figure 16. Diazonium preparation of 3-ferrocenylpyridine

An organolithium technique (183) was employed in the preparation of 2-ferrocenylpyridine (Figure 17). As with the diazonium technique, this preparation gave disappointing yields, and then only after extensive purification.

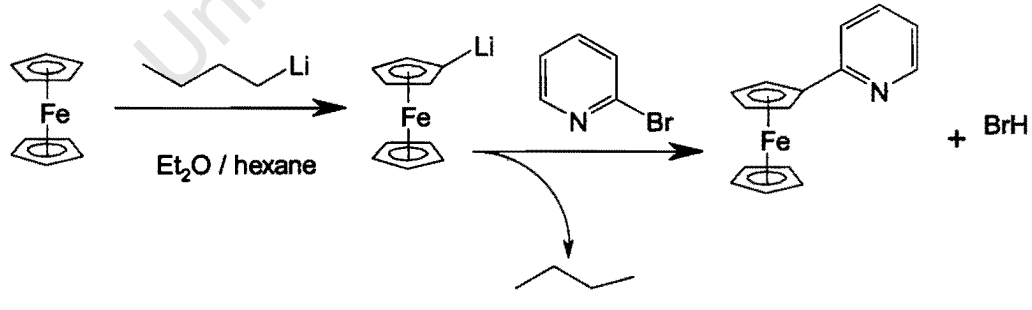

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Figure 17. Organolithium preparation of 2-ferrocenylpyridine

Attempted preparation of (4'-methoxyphenyl)pyridine

This ligand was targeted with the intention of creating bulkier and slightly more hydrophilic analogues of the phenylpyridine ligands used extensively in this work. One attractive feature of these compounds is that there is an established method (184) of preparing them via diazonium chemistry, the techniques for which were already well-known to the author because of their use in the preparation of 3-ferrocenylpyridine. Moreover, a relatively recent publication describes the simple separation of the various isomers produced via column chromatography (185). However, although fractions enriched in one-or-other isomer were easily obtained, no completely pure isomer was successfully isolated by chromatography.

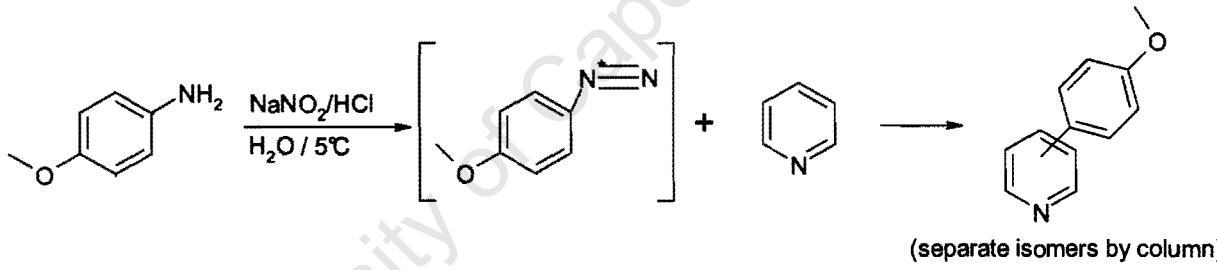


Figure 18. Attempted preparation of (4'-methoxyphenyl)pyridine , via diazonium chemistry

Preparation of metal complexes

Preparation of gold(I) complexes

In this work, several linear complexes of gold(I) have been prepared. This metal centre is usually accessed via the reduction of a gold(III) species with a thioether ligand. This convention has been followed, with the preparation of

tetrahydrothiophene gold(I) chloride from auric acid (Figure 19, A). The tetrahydrothiophene gold(I) pentafluorophenyl starting material is obtained from this compound via anion replacement, using an organolithium reagent. (Figure 19, B) Following the protocol of Barranco et al (117), (3-ferrocenylpyridine)gold(I) chloride has been prepared, although in somewhat low yield, most likely due simply to the small scale on which the reaction was attempted and consequent transfer losses.

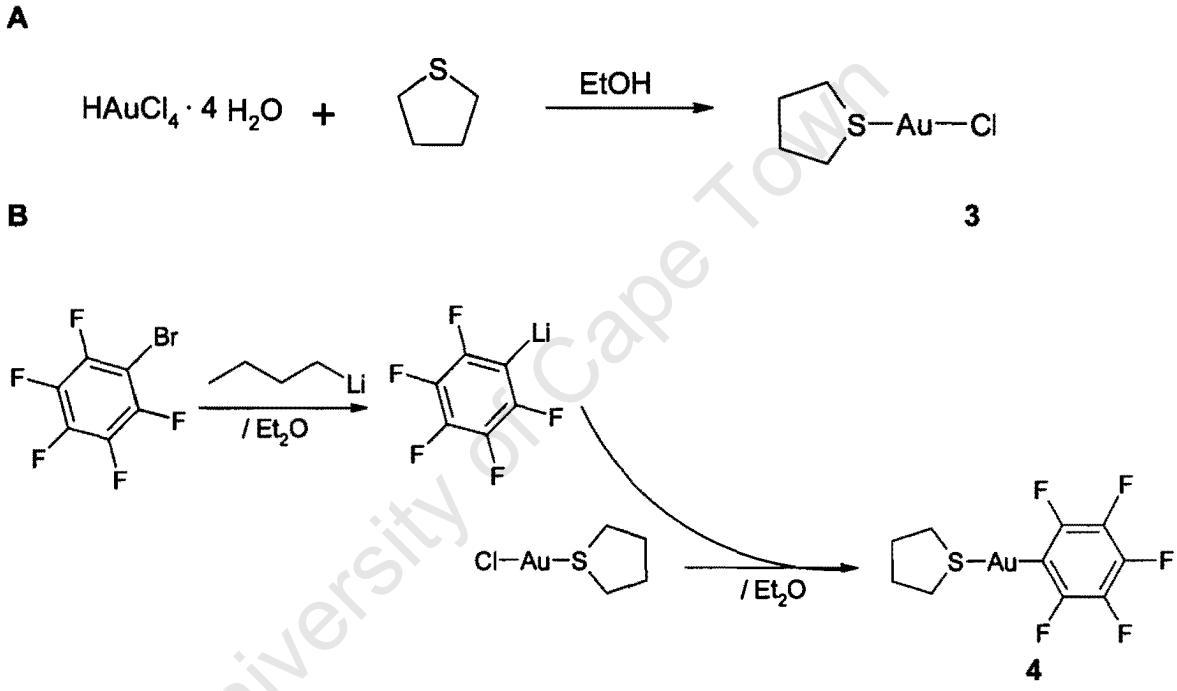


Figure 19. Preparation of (THT)gold(I) chloride (**A**) and (THT)gold(I) pentafluorophenyl (**B**)

Despite following this method closely, the preparation of the corresponding phenylpyridine complexes has been considerably more difficult. This is most likely because of the preference of the gold(I) centre for "softer" ligands, as noted above. Whereas the ferrocenyl substituent of ferrocenylpyridine is somewhat electron-withdrawing, a phenyl substituent is electron-donating,

leading to increased electron density on the pyridine nitrogen. Thus the metal centre is presented with a "hard" ligand which contributes to instability.

As noted in the introduction, above, gold(I) species readily disproportionate into gold(III) and metallic gold. Unfortunately this problem of stability has been a very significant challenge in preparing pure phenylpyridine gold complexes. These products are unstable in those solvents in which they are soluble, presumably because the disproportionation takes place more readily if the molecules of the complex are able to come into contact with each other, as in solution. While a more detailed study of the stability of these complexes in various solvents is outside the scope of this study, it is worth noting that the process of disproportionation was more rapid in more polar solvents. Hence colloidal gold is formed virtually instantaneously upon contact with acetone or DMSO, and over the course of a few minutes in chloroform. Nevertheless, if care is taken, these complexes do exist in chloroform for long enough to acquire NMR spectra.

In dichloromethane, by way of contrast, colloidal gold is not observed to precipitate for several hours. Thus this has been the preferred solvent for complex formation. Nonetheless, each reaction must be timed carefully to avoid decomposition. Also, after the reaction is complete, the solvent must be removed *in vacuo* and the product washed with diethyl ether and then thoroughly dried. Two of the three potential isomers of (phenylpyridine)gold(I) chloride have been prepared in this way, and been satisfactorily characterised. With knowledge of the literature, some attempt can be made to rationalise the differing stability of the various complexes. It is known that the gold(I) centre, in general, has greater affinity for "softer" electron donors. Since the ferrocene moiety of ferrocenylpyridine is expected to be a net acceptor of electron density, we expect the pyridine nitrogen of this ligand to be a considerably weaker electron donor than the corresponding nitrogen of phenylpyridine, where the benzene moiety contributes to increased electron density. This accounts for the relative stability of complexes of gold(I) with soft ferrocenylpyridine ligands (and

thus, their presence in the existing literature), and the corresponding instability of phenylpyridine complexes.

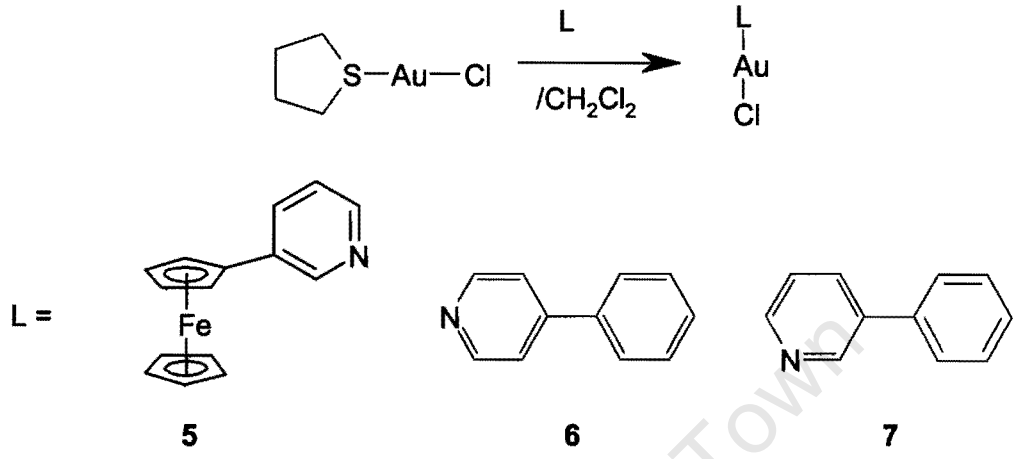


Figure 20. Preparation of pyridine based complexes with gold(I) chloride

(Phenylpyridine)gold(I) pentafluorophenyl complexes generally exhibit greater stability in solution, forming a fine purple precipitate of colloidal gold only after some hours in chloroform. Each of the three potential isomers of this compound has therefore been prepared in high purity (Figure 21). The yield for each of these preparations remains low (approximately 10-50 %), partially because of the small scale, but also because these complexes are slightly soluble in diethyl ether, resulting in material being lost during the washing phase. Better yields might perhaps have been achieved by using chilled diethyl ether.

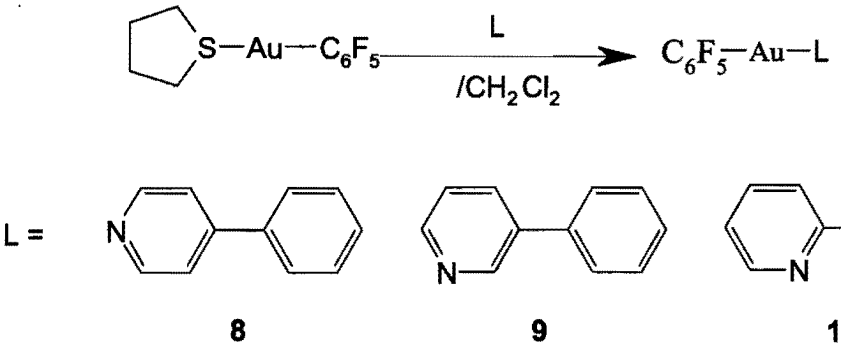
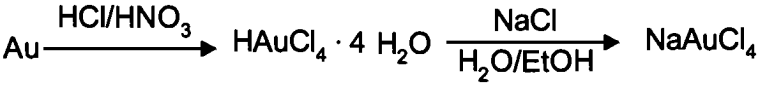


Figure 21. Preparation of pyridine based complexes with gold(I) pentafluorophenyl

Preparation of gold(III) complexes

Gold in the 3+ oxidation state is obtained by the oxidising effect of *aqua regia*, a mixture of nitric and hydrochloric acid known by mediaeval alchemists to dissolve gold. (110) The product of this oxidation is auric acid, which is easily converted to sodium tetrachloroaurate. (Figure 22) Both of these species are here used as starting materials. The first preparation of (pyridine)gold(III) trichloride is dated to the beginning of the twentieth century, so the chemistry of this sort of compound is relatively well-understood. The method of Colles and Gibson (135), dating to 1931, has been used in preparing this compound. (Figure 23) This method gives a product in good yield, and with characterisation data matching expectations. However, for the phenylpyridine complexes of gold(III), it was decided to use the experimental conditions cited for the preparation of (2-phenylpyridine)gold(III) trichloride by Constable and Leese (186). This method gave pure yields generally in excess of 70 %. (Figure 24) A complete set of geometric isomers of (phenylpyridine)gold(III) trichloride was prepared via this method. The cycloaurated compound (2-phenylpyridine)gold(III) dichloride was also prepared by direct reaction (Figure

25), as transmetallation techniques were felt to be unnecessarily complicated and hazardous.



11 **12**

Figure 22. Preparation of gold(III) starting materials

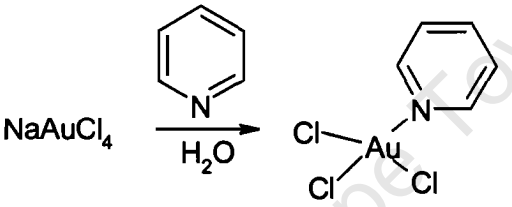


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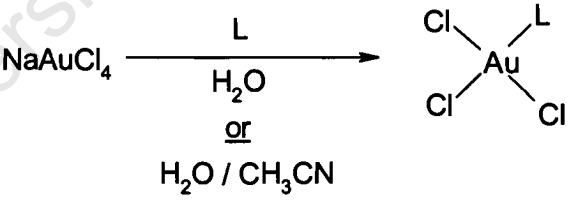
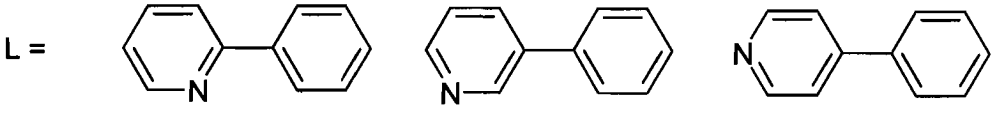
Figure 23. Preparation of (pyridine)gold(III) trichloride





14 **15** **16**

Figure 24. Preparation of (phenylpyridine)gold(III) trichloride

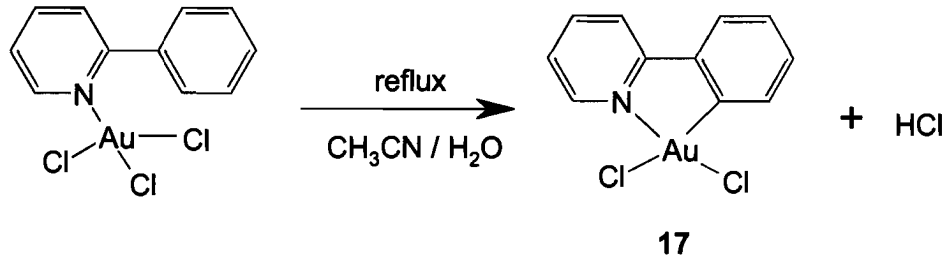


Figure 25. Preparation of (2-phenylpyridine)gold(III) dichloride

Hijazi et al (187) present a very intriguing piece of work. By simple reflux of a ruthenium starting material with a 2-phenylpyridine derivative, they are able to form a C,N-donor ruthenocycle. This methodology follows directly from the syntheses described above. However, they then go on to carry out the analogous reaction with 2-ferrrocenylpyridine, yielding a C,N-ruthenocycle with ferrocene in the ring. This work suggests that it might be stereochemically feasible to form an analogous 2-ferrocenylpyridine aurocycle, although no precedent exists for this in the literature. Ferrocene carbon-gold σ-bonds have been demonstrated, but only via lithiation of ferrocene to form a strong nucleophile (188,189).

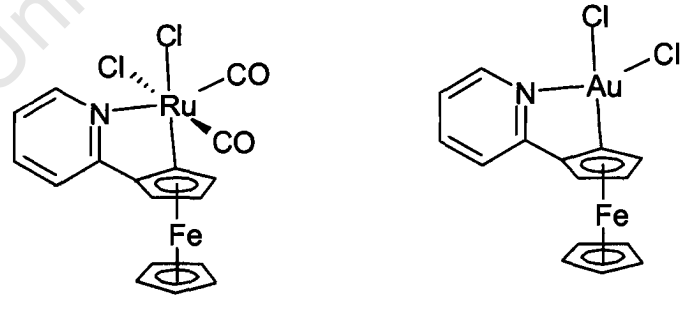


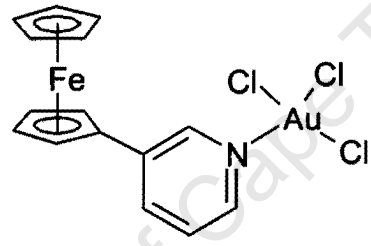
Figure 26. Literature ruthenium(II) compound, and suggested gold(III) analogue

Following the protocol taken from Constable and Leese, preparation of the 2-ferrocenylpyridine aurocycle first requires the N-donor complexation of the pyridine moiety. Ferrocenylpyridine analogues of the phenylpyridine complexes were thus attempted via this protocol, yielding bright green solids, in contrast to the shades of yellow usually encountered with complexes of gold(III). Interestingly, and in strong contrast to the phenylpyridines, it was found that these complexes were characterised by marked instability. These compounds appeared to break down very rapidly into colloidal gold in solution, and rather more slowly in aqueous suspension. Moreover, it was found that addition of excess ligand during complex formation similarly resulted in the formation of a fine black precipitate consistent with colloidal gold. This response was similarly elicited when ferrocenylpyridine was added to the gold(III) starting material in solution of acetonitrile, as was done with phenylpyridine, presumably due to excess local concentration that may occur in even a well-stirred mixture. This necessitated that solid ferrocenylpyridine be added directly to an aqueous solution of tetrachloroaurate ion, resulting in a relatively slow reaction yielding apparently pure product.

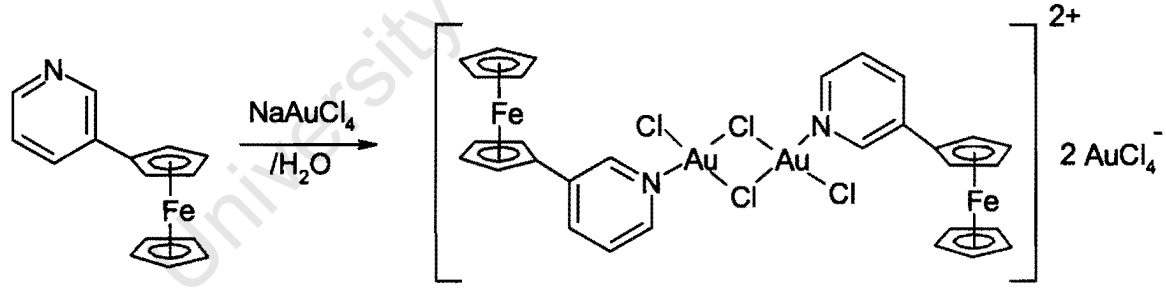
This problem of instability meant that the cycloaurated complex of 2-ferrocenylpyridine was unlikely to be achieved, although a transmetallation methodology may overcome this difficulty. Nevertheless, the unexpected properties of the green complexes represented an interesting problem in itself. Because of the instability of these products, isolation and characterisation represented a significant challenge. However, elemental analysis was performed successfully in the case of the 3-ferrocenylpyridine isomer. The same analysis was not performed for the 2-ferrocenylpyridine isomer but, since it is similar to the product under discussion, in both appearance and the physical properties noted above, it is reasonable to assume that following discussion will apply here also. Despite the apparent purity of the 3-ferrocenylpyridine product, as assessed by the colour and texture of the solid, this analysis did not yield data consistent with the complex (3-ferrocenylpyridne)gold(III) trichloride (see Figure

27, A). These data were, however, consistent with the formula (3-ferrocenylpyridne)gold(III) *di*chloride *tetrachloroaurate*. Since gold in the 3+ oxidation state is generally found to have 4-coordinate geometry, this formula would necessitate the formation of a chloro-bridged gold dimer, as depicted in Figure 27, B, below. This sort of structure is known in the literature (158,190,191), usually in the context where the incoming ligand is an anion. However, given the lack of further rigorous analytical data supporting this structure, it must remain a tentative proposal. Nevertheless, several pieces of observational evidence do support the hypothesis, as described below.

A



B



18

Figure 27. Original proposed structure for the gold(III) complex of 3-ferrocenylpyridine (A) and structure hypothesised in the light of elemental analysis data (B)

Firstly, it should be noted that the physical properties of the product are substantially different to those of the corresponding phenylpyridines, including colour but especially its far greater instability. As noted above, this compound proved highly unstable in solution (of both non-halogenated organic polar solvents and chlorinated solvents). Moreover, the slow decomposition of the complex in aqueous suspension is markedly increased in contact with metal – black decomposition product is observed to form on a metal spatula used in processing the reaction product, for instance. This last observation might lead to the conjecture that decomposition involves a redox reaction that is perhaps catalysed by the availability of free electrons in a metal. The second interesting observation is that the product is noticeably more stable in aqueous solution containing tetrachloroaurate ion than in pure water. This is clear from the way in which product that has been collected by filtration and washed by pure water decomposes much more rapidly than product from the same batch that has remained standing in the mother liquor. This suggests that the dissociation of tetrachloroaurate ion is a crucial step in decomposition, as it is this step that would be disfavoured by a high concentration of the ion in the surrounding medium. This may also account for the generally instability of the product in solution, especially with polar solvents, as these solvents are more likely to bring about ionic dissociation.

Despite the instability of the product in solution, an NMR spectrum (Figure 28) was recorded in deuterated acetone, the solvent in which visible decomposition appeared to be slowest. The only peaks of interest were of very small magnitude and were quite broad, occurring at 9.23 and 8.43 ppm. Despite the poor quality of these data, the position of these peaks is consistent with a pyridine moiety bonded to a strongly electron-withdrawing metal centre, as this tends to produce a downfield shift. For instance, the most downfield pyridine signal for 4-phenylpyridine occurs at 8.66 ppm, whereas the corresponding signal for (4-phenylpyridine)gold(III) trichloride occurs at 9.29 ppm.

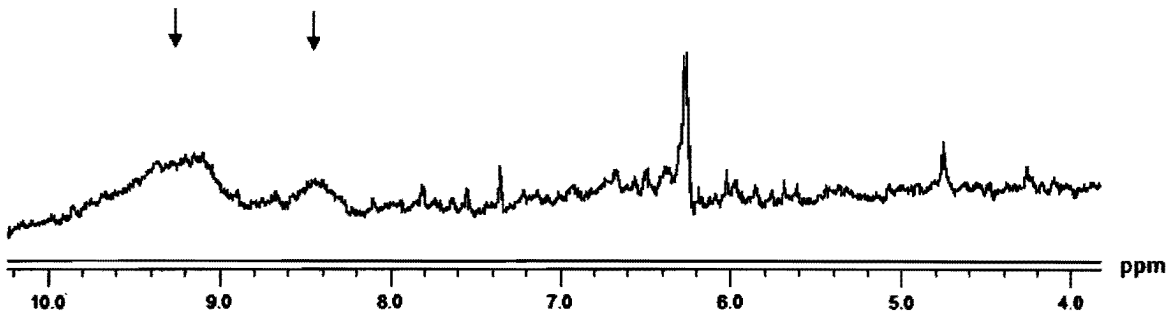


Figure 28 ^{1}H NMR spectrum of the 3-ferrocenylpyridine complex of gold(III), recorded in acetone-d6

Finally, an infrared spectrum was determined for the product (Figure 29). Altogether, the measured absorption peaks largely match those previously observed for 3-ferrocenylpyridine (88). However, the spectrum for **18** also contains a variety of signals at wave numbers of 355 cm^{-1} and lower, which are very likely due to Au-Cl bonds. Following Adam and Churchill's (192) characterisation of the most basic chloro-bridged gold dimer, Au_2Cl_6, we can assign these signals more specifically. The series of peaks in the 355-303 cm^{-1} region correspond to the stretching mode of Au-Cl bonds, for either terminal or bridging chlorides. Interestingly, the spectrum shown here has a distinct signal at 295 cm^{-1}, which matches closely a 291 cm^{-1} signal assigned by these authors to a bridge stretching mode. This observation provides further support to the hypothesis that the reaction product of 3-ferrocenylpyridine with tetrachloroaurate ion is the dimeric compound depicted in Figure 27, B.

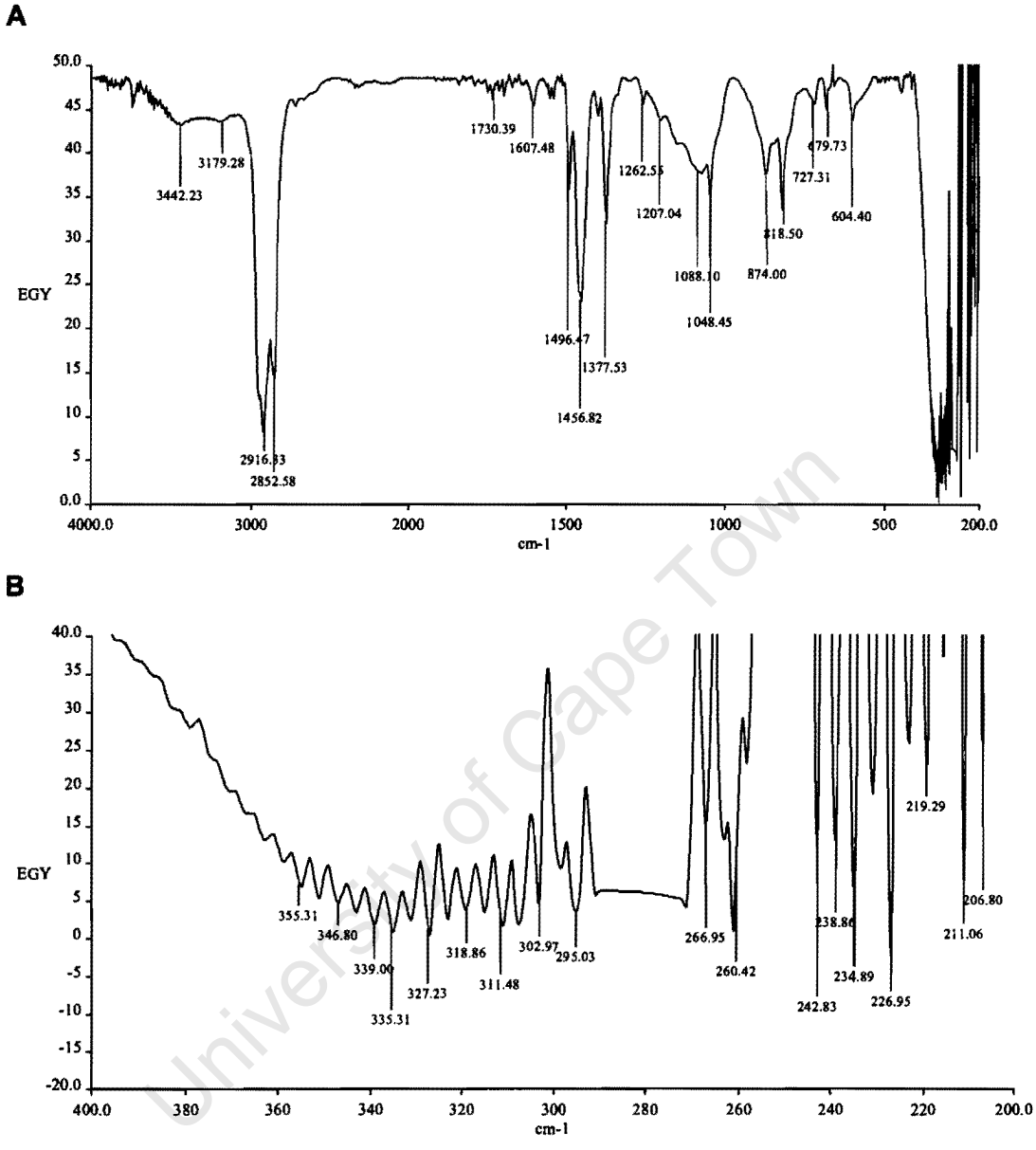
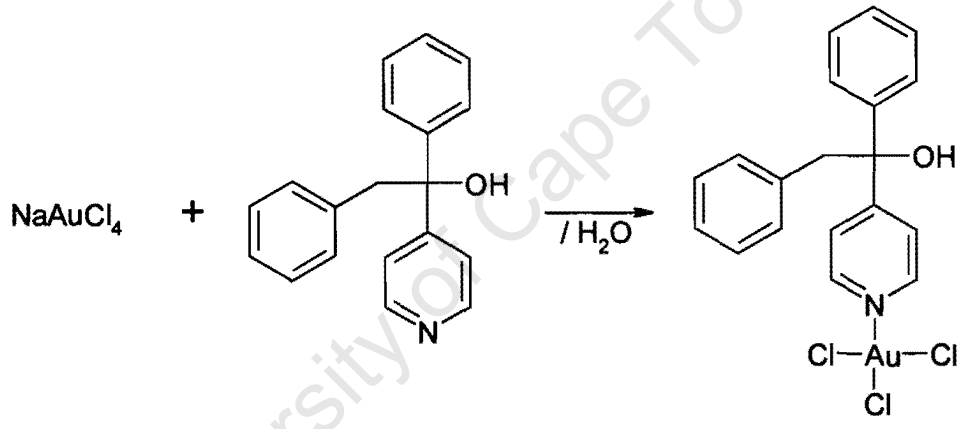


Figure 29. IR spectrum for **18**. (A) The complete spectrum; (B) a magnification of the 400-200 cm^{-1} region, containing the majority of peaks corresponding to Au-Cl.

The one other pyridine-based ligand used for these studies was hydroxy(phenylmethyl)-phenylpyridylmethane. Complexation was accomplished

by addition of solid ligand directly to an aqueous solution of tetrachloroaurate (Figure 30), this method being used mainly because the ligand was insoluble in acetonitrile and other water-miscible solvents. Complex-formation was observed in the gradual colour change of the precipitate from white to yellow, and the corresponding removal of colour from the supernatant. The relatively slow reaction time observed here, in comparison with phenylpyridine or ferrocenylpyridine was most probably due to the fact that this ligand has both an acidic hydroxyl moiety and basic pyridyl group, meaning that it is likely to form a zwittterion at neutral pH. Thus, at equilibrium, only a small proportion of ligand molecules will have the reactive pyridine nitrogen available for complexation.



19

Figure 30. Preparation of (hydroxy(phenylmethyl)-phenylpyridylmethane) gold(III) trichloride

Preparation of rhodium(I) complexes

The basic starting material used for most reactions was the μ-chloro(1,5-cyclooctadiene)rhodium(I) dimer. This has the advantage of being

straightforward to prepare, stable in air at room temperature, and easy to work with. The basic synthetic path for all pyridine N-donor complexes of Rh(I) follows the method suggested by Rajput (90) and Fougeroux *et al* (87), namely stirring the ligand with the dimeric starting material in dichloromethane. Most complexes were obtained in high purity, as assessed by NMR, and yields ranging from 40 – 80 %. Elemental analysis revealed (1,5-cyclooctadiene)(2-ferrocenylpyridine)rhodium(I) chloride to be impure, in a fashion consistent with the presence of starting material in the product. This was most likely to the fact that the reaction was attempted on a small scale and with 1:1 stochiometry, a combination of factors that may have resulted in a small measurement error (i.e. of the mass of ligand) having a relatively large effect on the product. The presence of starting material is also suggested by "shoulders" in the ^{1}H NMR signals of the product corresponding to cyclooctadiene.

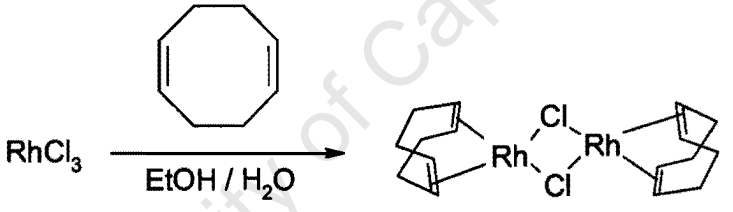


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Figure 31. Preparation of μ-chloro(1,5-cyclooctadiene)rhodium(I) dimer

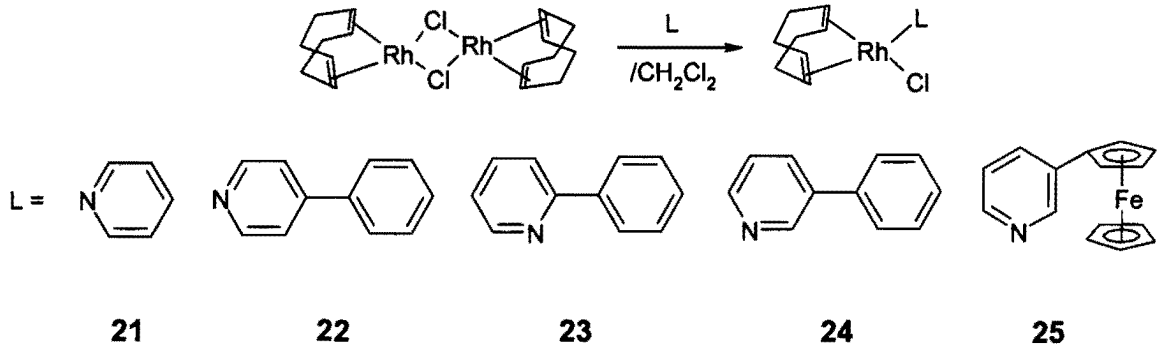

Figure 32. Preparation of pyridine based rhodium(I) complexes

Some reactions were also attempted with the chlorobis(cyclooctene)rhodium(I) dimeric starting material, which was prepared via an established literature method (193). (Figure 33) Although elemental analysis revealed that the starting material was somewhat impure, it was decided to react this dimer with pyridine and 4-phenylpyridine, yielding fine powders quite distinct in appearance from the dimer. The starting material and resulting products appear to be almost entirely insoluble in all organic solvents, so that reactions were carried out in toluene suspension with easy separation of products via filtration, as suggested by Milstein and co-workers (194,195). Although lack of solubility disallowed NMR analysis of the starting material, the products of reaction with pyridine containing ligands were found to be very slightly soluble in chloroform. The NMR spectra consequently obtained were found to contain peaks in the aromatic region, suggesting successful complexation with these ligands, although the peaks were too faint to allow reliable proton quantitation. The elemental analysis data obtained for these products were characterised by much lower carbon and hydrogen, and higher nitrogen, composition than would be expected if complex formation proceeded via a bridge-splitting reaction like that seen with the 1,5-cyclooctadiene starting material. It seems likely that, with the cyclooctene

starting material, this type of reaction is outcompeted by displacement of the alkene by the N-donor ligands (see Figure 34).

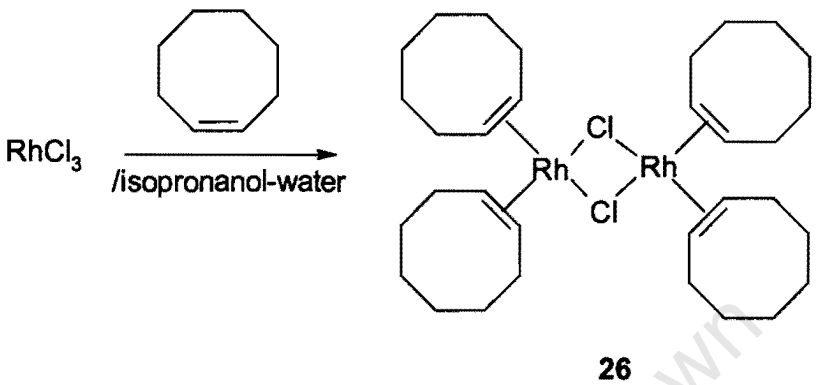

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Figure 33. Preparation of chlorobis(cyclooctene)rhodium(I) dimer

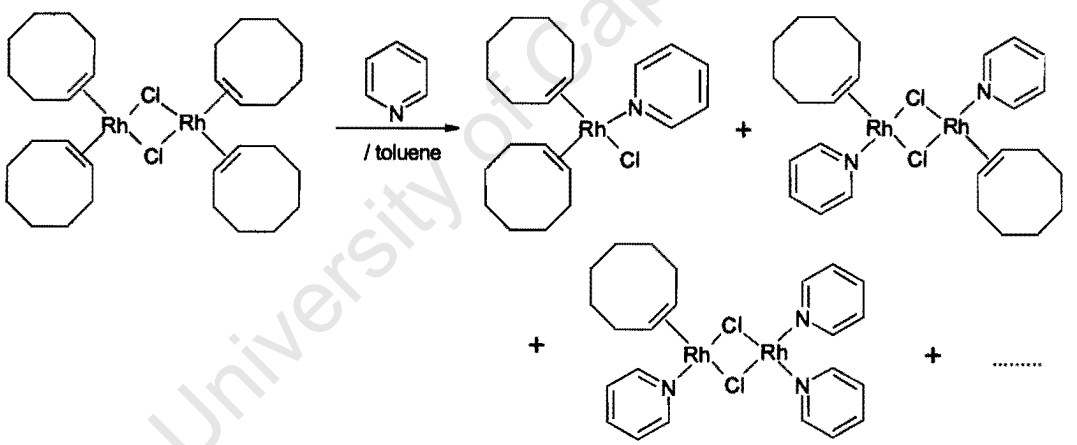


Figure 34. Hypothesised reaction of pyridine with chlorobis(cyclooctene) rhodium(I) dimer

Experimental

General experimental conditions

Syntheses were carried out in air, unless otherwise stated. Were oxygen-free conditions were required, syntheses were performed under prepurified nitrogen gas, using standard Schlenk techniques. Deionised water (dH_2O) was prepared by filtration through an ion exchange resin. All solvents were used as received, except where Schlenk techniques were applied, or otherwise indicated. Where necessary, diethyl ether was distilled under nitrogen from Na/benzophenone/tetraglyme prior to use, then stored under nitrogen (196). Ethanol and acetone (Merck) were used as received. 0.10 M HCl was obtained from Cameron Chemical Consultants, Clareinch, South Africa. All other reagents were obtained from Sigma-Aldrich and used as received, unless otherwise stated. Thin-layer chromatography was carried out on silica plates (Machery-Nagel, 0.20 mm Alugram SIL G/UV_{254}). Column chromatography was carried out using silica gel (Merck, Silica Gel 60). Melting point experiments were carried out on a Kofler hot-stage microscope (Reichert Thermovar), with the sample pressed between glass coverslips. Elemental analyses were conducted with a Carlo Erba EA1108 apparatus. NMR spectra were obtained with either a Varian Unity-400 (H: 400 MHz; C: 100.6 MHz) or a Varian Mercury-300 (H: 300 MHz; C: 75.5 MHz) spectrometer, both at the University of Cape Town. Infrared spectra were determined on a Perkin-Elmer Spectrum One FT-IF Spectrometer, with the sample prepared in a Nujol mull pressed between two sodium chloride plates. Each sample was scanned twelve times and the final spectrum taken as the average.

Preparation of ligands

*Preparation of 3-pyridinylferrocene (**1**)*

This was prepared via a modification of a previously reported technique (197). 3-Aminopyridine (4.706 g, 50 mmol) was dissolved in a mixture of dH_2O and HCl_{conc} (1:1) (40 ml) and cooled in an ice bath. $NaNO_2$ (3.795 g, 55 mmol) in ice-cold dH_2O (20 ml) was added dropwise to this. This resulted in a colour change of the solution from pink to yellow, indicating the formation of the diazonium salt of 3-aminopyridine. The diazonium salt was then added slowly, with stirring, to ferrocene (9.302 g, 50 mmol) in glacial acetic acid (350 ml), at room temperature. Gas was observed to evolve from the reaction mixture, which was allowed to stir overnight. After completion of the reaction, the mixture was basified by addition of NaOH (0.10 M), which caused the precipitation of solid material. The pH was monitored using Merck Universal Indicator strips. The precipitate was collected by centrifugation (2000 rpm, 10 min) and the reddish supernatant discarded. Product was recovered from the precipitate by exhaustive diethyl ether extraction. The ether fractions were dried over $MgSO_4$, then removed by rotary evaporation. The product was then redissolved in minimal n-hexane. 3-Pyridinylferrocene was confirmed to be present in the sample by thin-layer chromatography (R_f = 0.55 in Et_2O). The solution was loaded onto a silica column (Merck, Silica Gel 60) and unreacted ferrocene eluted as an orange band with n-hexane. 3-Pyridinylferrocene was not mobile in n-hexane, but eluted as a deep red band upon addition of diethyl ether to the column. The collected fraction was dried with rotary evaporation to yield 3-pyridinylferrocene as orange granules (2.263 g, 8.60 mmol, 17 %).

Properties:

Fine orange crystals at room temperature. Air stable.

Characterisation:

^{1}H NMR: ($CDCl_3$, 300.76 MHz) 8.74 (s, 1H), 8.42 (d, 1H), 7.7 (d, 1H), 7.20 (dd, 1H), 4.6 (t, 2H), 4.36 (t, 2H), 4.05 (s, 5H). Good agreement with literature (198).

m.p.: 58-61℃ (lit: 56-58℃ (199)).

*Organolithium preparation of 2-ferrocenylpyridine (**2**)*

This was prepared according to a literature method (200). Ferrocene (3.724 g, 20 mmol) was added to dry diethyl ether (80 ml), under a nitrogen atmosphere. n-Butyllithium (20 ml of a 1.6M solution in hexane, 32 mmol) was added to this. This mixture was stirred, under nitrogen, for approximately four days. Unfortunately, due to defective equipment, this was partially exposed to atmospheric oxygen. Nevertheless, to this reaction mixture was added 2-bromopyridine (1.19 ml, 12.5 mmol) in dry diethyl ether (20 ml). After overnight stirring, approximately 10 ml of dH_2O was added, to eliminate any unreacted organolithium reagents. To this was added a small quantity of aqueous NaOH (40 %), to ensure maximum partitioning of the basic product into the organic phase. The organic phase was washed several times with dH_2O, then collected and solvent removed by rotary evaporation. The two major components of the product mixture were identified as ferrocene (R_f = 0.95, in diethyl ether) and 2-ferrocenylpyridine (R_f = 0.85, in diethyl ether) by thin-layer chromatography.

The dry product mixture was redissolved in n-hexane fractions and loaded onto an alumina column (Merck, aluminium oxide 90, active neutral; deactivated by addition of dH_2O). Thin-layer chromatography revealed that only ferrocene and 2-ferrocenylpyridine eluted in hexane. The collected hexane fractions containing these compounds were loaded onto a silica column (Merck, Silica Gel 60). Ferrocene eluted with hexane, as was some 2-ferrocenylpyridine, due to

overloading of the gel. Comparatively pure 2-ferrocenylpyridine was eluted with diethyl ether, as confirmed by thin-layer chromatography. Solvent was removed under high vacuum, yielding deep red crystals (0.522 g, 1.98 mmol, 10 %)

Properties:

Sticky, deep red solid at room temperature.

Characterisation:

^{1}H NMR: ($CDCl_3$, 400 MHz) δ: 8.50 (d, 1H), 7.57 (t, 1H), 7.41 (d, 1H), 7.06 (t, 1H), 4.92 (t, 2H), 4.40 (t, 2H), 4.05 (s, 5H). Good agreement with literature. (201)

Attempted preparation of (4'-methoxyphenyl)pyridine

(Methoxyphenyl)pyridine isomers were prepared according to a literature method (184,202). *p*-Anisidine (2.470 g, 20 mmol) was dissolved in a mixture of water (20 ml) and concentrated hydrochloric acid (20 ml). This was cooled to approximately 0-5℃ in an ice bath, whereupon sodium nitrite (1.513 g, 22 mmol) in water (10 ml) was added, with stirring. The resulting diazonium salt solution was added over the course of 150 min to pure pyridine (4 ml, 3.92 g, 49.6 mmol), with stirring at 70-80℃. The mixture was stirred for a further 2 h at 80℃. The mixture was neutralised with concentrated ammonia solution and heated over a steam bath to allow the removal of pyridine.

The resulting oil solution was extracted with benzene, which was removed under vacuum to yield the product mixture. The different product isomers were separated on a silica gel column with 1:1 ethyl acetate/hexane, and the components of each fraction determined by thin-layer chromatography. No fraction gave a sufficiently pure enrichment of any isomer to warrant further purification.

Preparation of gold(I) compounds

Preparation of tetrahydrothiophene gold(I) chloride (3)

This was prepared according to a well-established literature method (203). An aliquot of $HAuCl_4$ stock solution (20 ml, 11.9 mmol) was added to ethanol (40 ml). To this mixture was added liquid tetrahydrothiophene (THT), dropwise. Addition was continued, with stirring, until the yellow colour had faded from solution. The white precipitate was filtered off and washed with dH_2O. The clumpy white solid was dried under high vacuum (3.396 g, 10.6 mmol, 89 %, calculated from $HAuCl_4$).

Properties:

Coherent, granular white solid. Decomposes slowly to a faintly pink solid at room temperature, but quite stable at 4℃.

Characterisation:

^1H NMR ($CDCl_3$, 300.1 MHz) δ: 3.42 (br s, 2H), 2.19 (br s, 2H). Good agreement with literature. (203)

Found:	C, 15.06%; H, 2.48%; S, 10.10%.
Calc for C_4H_8SAuCl:	C, 14.98%; H, 2.52%; S, 10.00%.

m.p. Decomposes for temperature greater than approximately 95℃. Melts 124-142℃.

*Preparation of Tetrahydrothiophene gold(I)pentafluorophenyl (**4**)*

This was prepared according to a well-established method (203). A solution of bromopentafluorobenzene (0.263 ml, 0.51 g, 2.06 mmol) in degassed diethyl ether (20 ml) at -78℃ was treated with n-butyl lithium in hexane (2.06 mmol, 1.29 ml of 1.6 M solution). The solution was stirred for 1h upon which a suspension of Au(THT)Cl (0.656 g, 2.04mmol) in diethyl ether (40ml) was added. The colourless solution was stirred for 15 min at -78°C. The cooling system was allowed to warm gradually over the course of 45 min, after which it was removed. The mixture was then stirred for a further 2 h. Most solvent was then removed in vacuo to yield a white solid, with a brown substance remaining in solution. The solid was collected by filtration (0.5097 g, 1.13 mmol, 55 %)

Properties:

Fine white powder. Decomposes to a pink or purple solid, then to a brown solid, at room temperature, but quite stable at 4℃.

Characterisation:

Found:	C, 26.40%; H, 1.70%; S, 5.54%.
Calc. for $C_{10}H_8F_5SAu$:	C, 26.56%; H, 1.79%; S, 7.09%.

m.p. Decomposes from 95-113℃

*Preparation of (3-pyridinylferrocene)AuCl (**5**)*

This was prepared according to the method of Barranco *et al* (117). 3-Ferrocenylpyridine (26.2 mg, 0.1 mmol) was dissolved in dichloromethane (10 ml). (THT)AuCl (32.4 mg, 0.1 mmol) was dissolved in dichloromethane (10 ml) and added to the 3-ferrocenylpyridine. The mixture was stirred for 90 minutes,

then most of the solvent removed by rotary evaporation and a large excess of diethyl ether added. The resulting precipitate was collected by filtration and washed with diethyl ether (20.4 mg, 0.04 mmol, 41 %).

Properties:

The product was a fine orange powder, stable in air at room temperature.

Characterisation:

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.64 (s, 1H), 8.34 (d, 1H), 8.96 (d, 1H), 7.41 (t, 1H), 4.68 (br s, 2H), 4.51 (br s, 2H), 4.11 (br s, 5H). Good agreement with literature values. (117)

Found: C, 35.26%; H, 2.43%; N, 2.40%.

Calc. for $C_{15}H_{13}FeNAuCl$: C, 36.35%; H, 2.65%; N, 2.83%.

*Preparation of (4-phenylpyridine)AuCl (**6**)*

Solid 4-phenylpyridine (0.217 g, 1.398 mmol) was reacted with (THT)AuCl (0.103 g, 0.321 mmol) in the same way described above. (Yield: 0.095 g, 0.245 mmol, 76 %).

Properties:

The product was a fine brown powder, stable in air at room temperature. It is soluble in polar solvents such as acetone and DMSO, but highly unstable, yielding a colloidal gold precipitate immediately upon dissolution. It is insoluble in water, but unstable in the presence of moisture. The compound is soluble in dichloromethane and chloroform, but decomposes over the course of hours (for the former) or minutes (for the latter).

Characterisation:

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.59 (dd, 2H), 7.73 (dd, 2H), 7.68 (m, 2H), 7.56 (m, 3H).

Found: C, 34.09%; H, 2.26%; N, 3.26%.

Calc. for $C_{11}H_9NAuCl$: C, 34.08%; H, 2.35%; N, 3.61%.

*Preparation of (3-phenylpyridine)AuCl (**7**)*

3-Phenylpyridine (0.20 ml, 0.22 g, 1.40 mmol) was reacted with (THT)AuCl (0.102 g, 0.318 mmol) in the same way described above. (Yield: 0.060 g, 0.155 mmol, 49 %).

Properties:

The product was a fine white powder, stable in air at room temperature. It is soluble in polar solvents such as acetone and DMSO, but highly unstable, yielding a colloidal gold precipitate immediately upon dissolution. It is insoluble in water, but unstable in the presence of moisture. The compound is soluble in dichloromethane and chloroform, but decomposes over the course of hours (for the former) or minutes (for the latter).

Characterisation:

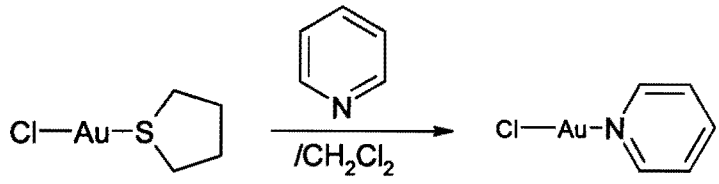
^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.83 (d, 1H), 8.55 (d, 1H), 8.18 (dt, 1H), 7.62 (dd, 1H), 7.54 (m, 5H).

Found: C, 33.92%; H, 2.31%; N, 3.31%.

Calc. for $C_{11}H_9NAuCl$: C, 34.08%; H, 2.35%; N, 3.61%.

m.p. Decomposes for temperature greater than approximately 99℃.

Attempted preparation of (pyridine)AuCl



This was prepared by a modification of a literature method. (115) Sufficient pyridine was added to completely cover a quantity of (THT)AuCl (0.080 g, 0.250 mmol). The resulting mixture was heated gently over a steam bath, until the solid was dissolved. This was heated for a further 30 min, whereupon a large excess of diethyl ether added. The precipitate was collected by filtration and washed with diethyl ether.

Properties:

The product is a fine white powder, which decomposes gradually in air at room temperature. It is soluble in polar solvents such as acetone and DMSO, but highly unstable, yielding a colloidal gold precipitate immediately upon dissolution. It is insoluble in water, but unstable in the presence of moisture. The compound is soluble in dichloromethane and chloroform, but decomposes over the course of hours (for the former) or minutes (for the latter).

Characterisation:

^{1}H NMR: Not performed, due to instability or insolubility in standard solvents

Found:	C, 19.41%; H, 1.61%; N, 4.23%.
Calc. for C_5H_5NAuCl:	C, 19.28%; H, 1.62%; N, 4.50%.

m.p.: Decomposition around 150℃, followed by melting from 220-250℃.

*Preparation of (4-phenylpyridine)gold(I)pentafluorophenyl (**8**)*

Solid 4-phenylpyridine (0.041 g, 0.27 mmol) was added to (THT)Au(C_6F_5) (0.123 g, 0.27 mmol) in dichloromethane (10 ml). The mixture was stirred for 50 minutes, after which most of the solvent was removed by rotary evaporation and an excess of diethyl ether added. The resulting precipitate was collected by filtration and washed with minimal diethyl ether to yield a fine white powder (0.0670 g, 0.13 mmol, 48 %)

Properties:

Fluffy white powder.

Characterisation:

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.67 (dd, 2H), 7.79 (dd, 2H), 7.70 (dd, 2H), 7.56 (m, 3H)

Found:	C, 39.24%; H, 1.73%; N, 2.17%.
Calc. for $C_{17}H_9F_5NAu$:	C, 39.32%; H, 1.75%; N, 2.70%.

*Preparation of (3-phenylpyridine)gold(I)pentafluorophenyl (**9**)*

3-phenylpyridine (0.040 ml, 0.043 g, 0.28 mmol) was added directly to (THT)Au(C_6F_5) (0.116 g, 0.257 mmol) in dichloromethane (10 ml). The mixture was stirred for 35 minutes, after which most of the solvent was removed by rotary evaporation and an excess of diethyl ether added. The resulting

precipitate was collected by filtration and washed with minimal diethyl ether to yield a fine white powder (0.012 g, 0.024 mmol, 9 %)

Properties:

Fluffy white powder.

Characterisation:

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.83 (d, 1H), 8.58 (dd, 1H), 8.14 (dt, 1H), 7.64 (dd, 1H), 7.51 (m, 5H)

^{19}F NMR ($CDCl_3$, 400 MHz) δ: -159.6 (d, 2F), -163.2 (m, 3F)

Found: C, 39.45%; H, 1.75%; N, 2.05%.

Calc. for $C_{17}H_9F_5NAu$: C, 39.32%; H, 1.75%; N, 2.70%.

*Preparation of (2-phenylpyridine)gold(I)pentafluorophenyl (**10**)*

2-phenylpyridine (0.040 ml, 0.043 g, 0.28 mmol) was added directly to (THT)Au(C_6F_5) (0.111 g, 0.25 mmol) in dichloromethane (10 ml). The mixture was stirred for 45 minutes, after which most of the solvent was removed by rotary evaporation and an excess of diethyl ether added. The resulting precipitate was collected by filtration and washed with minimal diethyl ether to yield a fine white powder (0.013 g, 0.03 mmol, 10 %)

Properties:

Fluffy white powder.

Characterisation:

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.76 (d, 1H), 8.03 (td, 1H), 7.91 (m, 1H), 7.73 (d, 1H), 7.52 (m, 5H)

Found:	C, 39.66%; H, 1.72%; N, 2.11%.
Calc. for $C_{17}H_9F_5NAu$:	C, 39.32%; H, 1.75%; N, 2.70%.

Preparation of gold(III) compounds

*Preparation of auric acid (**11**)*

This was prepared using a literature method (204). Gold metal (5.854 g, 29.7 mmol) was placed in freshly prepared aqua regia (HCl_{Conc}: HNO_{3Conc}; 3:1) (40ml) and stirred at room temperature until all gold particles had dissolved. Aqua regia was removed by evaporation over a steam bath at 70 ℃, yielding orange crystals of $HAuCl_4$. These were dissolved in a small amount dH_2O, which was also evaporated. The washed crystals were dissolved in dH_2O (50 ml), to be used as a stock solution.

*Preparation of sodium tetrachloroaurate (**12**)*

This was prepared using a literature method (204). Solid $HAuCl_4$ (8.674g, 21.06 mmol,) was redissolved in a mixture of water and ethanol (1:1) (40 ml) and solid NaCl (1.231g, 21.06 mmol) added directly to this, with overnight stirring. As the orange product was highly hydroscopic, solvent could not be entirely removed, even with heating under high vacuum. Therefore, the product was used to make a standard solution of $NaAuCl_4$ (21 mmol in 100 ml dH_2O).

*Preparation of (pyridine)$AuCl_3$ (**13**)*

$NaAuCl_4$ stock solution (5 ml of 21.0 mM stock, 1.05 mmol) was added to distilled water (10 ml). To this was added pyridine (0.12 ml, 0.12 g, 1.5 mmol), with stirring. A yellow solid was observed to precipitate out gradually over the course of 4 h stirring at room temperature. The product was collected by filtration, washed with distilled water and then a small amount of diethyl ether. The product was recovered as a bright yellow solid (0.435 g, 1.137 mmol, 108 %). The excess yield is most likely due to the accidental addition of too much tetrachloroaurate stock, resulting from measurement error.

Properties:

Soft yellow powder at room temperature. Air stable.

Characterisation:

^1H NMR (acetone-d, 400 MHz) δ: 9.13 (d, 2H), 8.43 (t, 1H), 8.00 (t, 2H).

Found: C, 15.61; H, 1.27; N, 3.00.

Calc. for $C_5H_5NAuCl_3$: C, 15.70; H, 1.32; N, 3.66.

m.p. Melts from 238-242℃.

*Preparation of (2-phenylpyridine)$AuCl_3$ (**14**)*

This compound was prepared according to literature (205). $NaAuCl_4$ stock solution (5 ml, 1.05 mmol) was added to dH_2O (15 ml). To this was added 2-phenylpyridine (0.129 ml, 0.14 g, 0.9 mmol) in acetonitrile (15 ml). With stirring, the yellow colour faded from the supernatant and a yellow precipitate appeared.

This was collected by filtration and washed with dH_2O. The fluffy, slightly yellow solid was dried under vacuum (0.358 g, 0.782 mmol, 74 %).

Properties:

Soft yellow powder at room temperature. Air stable.

Characterisation:

^{1}H NMR (acetone-d, 400 MHz) δ: 9.30 (d, 1H), 8.49 (td, 1H), 8.10 (dd, 1H), 8.03 (td, 1H), 7.91 (m, 2H), 7.69 (m, 3H). Good agreement with literature values. (206)

Found: C, 28.67; H, 1.08; N, 2.04.

Calc. for $C_{11}H_9NAuCl_3$: C, 28.81; H, 1.98; N, 3.05.

m.p. Melts from 170-175℃

*Preparation of (3-phenylpyridine)$AuCl_3$ (**15**)*

This compound was prepared according to a literature method (207). $NaAuCl_4$ stock solution (10 ml of 210 mM stock, 2.1 mmol) was added to distilled water (10 ml). To this was added 3-phenylpyridine (0.2 ml, 0.218 g, 1.40 mmol), with stirring. A yellow solid was observed to precipitate out gradually over the course of 4 h stirring at room temperature. The product was collected by filtration, washed with distilled water and then a small amount of diethyl ether. The product was recovered as a bright yellow solid (0.546 g, 1.192 mmol, 85 %).

Properties:

Soft yellow powder at room temperature. Air stable.

Characterisation:

^{1}H NMR (DMSO-d^6, 400 MHz) δ: 9.53 (d, 1H), 9.11 (br s, 1H), 8.77 (d, 1H), 8.61 (d, 1H), 7.95 (m, 1H), 7.90 (m, 2H), 7.54 (m, 2H).

Found:	C, 28.81; H, 1.84; N, 2.04.
Calc. for $C_{11}H_9NAuCl_3$:	C, 28.81; H, 1.98; N, 3.05.

m.p. Decomposes for temperatures greater than approximately 170℃. Decomposition characterised by formation of long filaments visible under light microscope.

*Preparation of (4-phenylpyridine)$AuCl_3$ **(16)***

This compound was prepared according to a literature method (208). Solid $KAuCl_4$ (0.265 g, 0.70 mmol) was dissolved in dH_2O (10 ml). To this was added 4-phenylpyridine (0.074 g, 0.48 mmol) in acetonitrile (10 ml). The mixture was stirred for 170 min, during which time the yellow colour faded from the supernatant and a yellow precipitate appeared. This was collected by filtration and washed with dH_2O and diethyl ether. Some impurities appeared in the product, so this was redissolved in acetone, filtered, and solvent removed under vacuum. The product was obtained as a fluffy, yellow solid. (0.1224 g, 0.27 mmol, 56 %).

Properties:

Soft yellow powder at room temperature. Air stable.

Characterisation:

^{1}H NMR (DMSO-d^6, 400 MHz) δ: 9.04 (d), 8.79 (s), 8.16 (dd), 7.92 (s), 7.58 (s).

Found: C, 28.84; H, 2.11; N, 2.59.

Calc. for $C_{11}H_9NAuCl_3$: C, 28.81; H, 1.98; N, 3.05.

m.p. Melts from 218-221℃, accompanied by some decomposition.

*Preparation of (2-phenylpyridine)$AuCl_2$ (**17**)*

Cycloauration was accomplished by the method of Constable and Leese (209). 2-Phenylpyridine gold(III) trichloride (0.230 g, 0.501 mmol) was suspended in aqueous acetonitrile (1:1, 40 ml) and heated under reflux for 4 hours. This resulted in the suspended solid becoming white and the supernatant becoming yellow in colour. The solid was collected by filtration and washed with dH_2O, then dried under vacuum (0.066 g, 0.15 mmol, 31 %).

Properties:

Soft off-white powder at room temperature. Air stable.

Characterisation:

^{1}H NMR (DMSO-d, 400 MHz) δ: 9.52 (d, 1H), 8.38 (dd, 1H), 7.95 (dd, 1H), 7.80 (dd, 1H), 7.75 (m, 1H), 7.46 (td, 1H), 7.37 (td, 1H). Good agreement with literature values.

Found: C, 29.67; H, 1.64; N, 2.80.

Calc. for $C_{11}H_8NAuCl_2$: C, 31.23; H, 1.91; N, 3.31.

*Preparation of complex of 3-ferrocenylpyridine with gold(III) (**18**)*

$NaAuCl_4$ (5 ml standard solution, 1.053 mmol) was placed in a beaker and the volume made up to 40 ml with dH_2O. Solid 3-pyridinylferrocene was added

directly to the mixture, gradually, with stirring. Although the ligand did not dissolve, it was observed that the surface of the solid became green and a green precipitate slowly accumulated. 3-Pyridinylferrocene was added gradually until the supernatant of the mixture became colourless. The mixture was then suction filtered and washed with water. The collected solid was washed with diethyl ether to remove water and then dried under high vacuum to yield a fine green powder (0.522 g, 0.92 mmol, 88 %). This decomposition was very rapid in the presence of a metal spatula, but not in the presence of glass or plastic implements.

Properties:

The compound is a very unstable bright green powder, which tends to decomposes to a brown solid. The compound is insoluble in non-polar solvents, such as diethyl ether. Contact with polar solvents (acetone, DMSO) and chlorinated solvents (dichloromethane, chloroform) gives rise to rapid decomposition, and the resulting brown solid appears to be soluble in these solvents. The product undergoes slow decomposition, which is quite rapid in the presence of water. This decomposition is slowed considerably in suspension of mother liquor containing tetrachloroaurate ion. Notably, this water-mediated decomposition occurs very rapidly when in contact with metal, such as a standard laboratory spatula, necessitating the use of glass implements in handling the product.

Characterisation:

Found:	C, 20.78; H, 1.59; N, 1.04.
Calc. for $C_{30}H_{26}Fe_2N_2Au_4Cl_{12}$:	C, 20.71; H, 1.51; N, 1.61.

IR (NaCl, cm^{-1}): 1730, 1607, 1496, 1263, 1207, 1088, 1048, 874, 819, 727, 680, 604, 355-303, 295, 267, 260, 243-219, 211, 207.

Preparation of complex of 2-ferrocenylpyridine with gold(III)

A small quantity of this compound was prepared, according to the method described above.

Properties:

Very unstable green powder, which appears to decompose to colloidal gold. Decomposition occurs slowly in air and rather more rapidly in aqueous suspension. Decomposition occurs very rapidly when in contact with metal (e.g. metal spatula), and in solution of polar solvents (acetone, DMSO) and chlorinated solvents (dichloromethane, chloroform).

Characterisation:

No characterisation data obtained, due to the instability of the product.

*Preparation of (1,2-diphenyl-1-pyridin-4-yl-ethanol)$AuCl_3$ (**19**)*

1,2-Diphenyl-1-pyridin-4-yl-ethanol (0.136 g, 0.494 mmol) was added as a white powder directly to a solution of $KauCl_4$ (0.230 g, 0.609 mmol) in water (10 ml). This was left to stir overnight, during which time the powder changed from white to yellow, and the golden colour faded from the supernatant. The solid was then collected by filtration and washed with diethyl ether to yield a yellow powder. (0.1443 g, 0.249 mmol, 51 %)

Properties:

Fine yellow powder. Stable at room temperature.

Characterisation:

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.67 (d, 2H), 7.65 (d, 2H), 7.50 (d, 2H), 7.40 (m, 3H), 7.21 (m, 3H), 6.91 (m, 2H), 3.48 (t, 2H).

^{13}C NMR: ($CDCl_3$, 400 MHz) δ: 163.00, 149.20, 143.06, 133.50, 130.57, 129.13, 128.64, 127.70, 126.03, 125.04, 77.42, 77.00, 76.57, 46.91)

Found: C, 39.38; H, 3.02; N, 1.97.
Calc. for $C_{19}H_{17}ONAuCl_3$: C, 39.43; H, 2.96; N, 2.42.

By contrast, the starting material:

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.49 (d, 2H), 7.46 (dt, 2H), 7.32 (m, 5H), 7.18 (m, 3H), 6.90 (m, 2H), 3.62 (m, 2H)

Preparation of rhodium(I) compounds

*Preparation of μ-chloro(1,5-cyclooctadiene)rhodium(I) dimer (**20**)*

This was prepared according to a literature method (210,211). $RhCl_3$ (2.005 g, 9.581 mmol) was dissolved in a mixture of water (5 ml), ethanol (25 ml) and a single drop of saturated Na_2CO_3 solution (deoxygenated by nitrogen bubbling). To this was added 1,5-cyclooctadiene (2.0 ml, 1.8 g, 16.3 mmol), and the mixture was refluxed under nitrogen. The solution was observed to become a deeper shade of red, and a yellow precipitate formed. After 15 h, more 1,5-cyclooctadiene (0.5 ml, 0.45 g, 4.1 mmol) was added and the reflux continued for further 3½ h. The precipitate was then collected by filtration, dissolved in minimal dichloromethane and recrystallised from dichloromethane/diethyl ether.

The product was obtained as fine orange-yellow crystals (1.552 g, 3.15 mmol, 66 %).

Properties:

Fine orange-yellow crystals at room temperature. Air stable.

Characterisation:

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.23 (bs, 4H), 2.50 (m, 4H), 1.75 (m, 4H). Good agreement with literature. (212)

Found:	C, 39.15%; H, 4.94%.
Calc. for $C_{16}H_{24}Rh_2Cl_2$:	C, 38.97%, H, 4.92%.

m.p. Melts from 226-231℃. (Lit: (213), 140-145℃; (90), 142-144℃)

*Preparation of (1,5-cyclooctadiene)(pyridine)rhodium(I) chloride (**21**)*

This was prepared according to Rajput's method (90). μ-Chloro(1,5-cyclooctadiene)rhodium(I) dimer (0.116 g, 0.471 mmol monomer) was dissolved in dichloromethane (10 ml), to which was added neat pyridine (0.04 ml, 0.04 g, 0.50 mmol), with stirring. The mixture was stirred for 45 min, after which the volume of dichloromethane was reduced under vacuum to approximately 5 ml and excess diethyl ether added. The resulting precipitate was collected by filtration and washed with diethyl ether. Bright yellow crystals were collected (0.066 g, 0.204 mmol, 43 %).

Properties:

Fine, bright yellow crystals at room temperature. Air stable.

Characterisation:

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.70 (d, 2H), 7.65 (t, 1H), 7.27 (t, 2H), 4.12 (br s, 4H), 2.46 (m, 4H), 1.79 (d, 4H). Good agreement with literature. (87)

Found: C, 46.10%; H, 5.04%; N, 3.88%.
Calc. for $C_{13}H_{17}NRhCl$: C, 47.95%; H, 5.27%, N, 4.30%.

m.p. Melts from 222-226℃. (Lit: (90), 230-233℃)

*Preparation of (1,5-cyclooctadiene)(4-phenylpyridine)rhodium(I) chloride (**22**)*

This was prepared as described above. μ-Chloro(1,5-cyclooctadiene)rhodium(I) dimer (0.123 g, 0.499 mmol monomer) and 4-phenylpyridine (0.080 g, 0.52 mmol) were dissolved in dichloromethane (10 ml), and stirred for 30 min, after which the volume of dichloromethane was reduced under vacuum to approximately 5 ml and excess diethyl ether added. The resulting precipitate was collected by filtration and washed with diethyl ether. Bright yellow crystals were recovered (0.1433 g, 0.357 mmol, 72 %).

Properties:

Fine, bright yellow crystals at room temperature. Air stable.

Characterisation:

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.76 (dd, 2H), 7.59 (m, 2H), 7.49 (m, 5H), 4.20 (bs, 4H), 2.52 (m, 4H), 1.85 (d, 4H). Good agreement with literature. (90)

Found: C, 56.90%; H, 5.35%; N, 3.33%.

Calc. for $C_{19}H_{21}NRhCl$: C, 56.80%, H, 5.28%; N, 3.49%.

m.p. Melts from 163-169℃.

*Preparation of (1,5-cyclooctadiene)(2-phenylpyridine)rhodium(I) chloride (**23**)*

This was prepared as described above. μ-Chloro(1,5-cyclooctadiene)rhodium(I) dimer (0.168 g, 0.681 mmol monomer) and 2-phenylpyridine (0.1 ml, 0.11 g, 0.70 mmol) were dissolved in dichloromethane (10 ml) and the mixture stirred for 40 min. The volume of dichloromethane was reduced under vacuum to approximately 5 ml and excess diethyl ether added. The resulting precipitate was collected by filtration and washed with diethyl ether. Bright yellow crystals were recovered (0.105 g, 0.26 mmol, 38 %).

Properties:

Fine, bright yellow crystals at room temperature. Air stable.

Characterisation:

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.70 (d, 1H), 8.42 (d, 1H), 7.99 (d, 2H), 7.73 (m, 2H), 7.47 (m, 2H), 7.23 (m, 1H), 4.23 (bs, 4H), 2.48 (bs, 4H), 1.75 (d, 4H).

Found: C, 53.00%, H, 5.27%, N, 2.53%.

Calc. for $C_{19}H_{21}NRhCl$: C, 56.80%, H, 5.28%; N, 3.49%.

m.p. Melts from 147-150℃.

*Preparation of (1,5-cyclooctadiene)(3-phenylpyridine)rhodium(I) chloride (**24**)*

This was prepared as described above. μ-Chloro(1,5-cyclooctadiene)rhodium(I) dimer (0.141 g, 0.572 mmol monomer) and 3-phenylpyridine (0.1 ml, 0.11 g, 0.70 mmol) were dissolved in dichloromethane (10 ml) and the mixture stirred for 45 min. The volume of dichloromethane was reduced under vacuum to approximately 5 ml and excess diethyl ether added. The resulting precipitate was collected by filtration and washed with diethyl ether. Bright yellow crystals were recovered (0.185 g, 0.460 mmol, 81 %).

Properties:

Fine, bright yellow crystals at room temperature. Air stable.

Characterisation:

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.96 (d, 1H), 8.70 (dd, 1H), 7.87 (dt, 1H), 7.60-7.40 (m, 5H), 7.37 (t, 1H), 4.20 (br s, 4H), 2.50 (br s, 4H), 1.84 (d, 4H).

Found: C, 56.82%; H, 5.34%; N, 3.34%.

Calc. for $C_{19}H_{21}NRhCl$: C, 56.80%, H, 5.28%; N, 3.49%.

m.p. Melts from 150-154℃.

*Preparation of (1,5-cyclooctadiene)(3-ferrocenylpyridine)rhodium(I) chloride (**25**)*

This was prepared as described above. μ-Chloro(1,5-cyclooctadiene)rhodium(I) dimer (0.025 g, 0.1 mmol) and 3-ferrocenylpyridine (0.029 g, 0.1 mmol) were dissolved in dichloromethane (10 ml) and the mixture stirred for 40 min. The volume of dichloromethane was reduced under vacuum to approximately 5 ml and excess diethyl ether added. The resulting precipitate was collected by

filtration and washed with diethyl ether. Bright red crystals were recovered (0.021 g, 0.04 mmol, 40 %).

Properties:

Bright red crystals at room temperature. Air stable.

Characterisation:

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.80 (d, 1H), 8.52 (d, 1H), 8.71 (dt, 1H), 7.20 (dd, 1H), 4.67 (t, 2H), 4.41 (t, 2H), 4.08 (s, 5H), 2.52 (br s, 4H), 1.85 (d, 4H), 1.55 (br s, 4H). Good agreement with literature. (59)

Found: C, 54.09%; H, 4.92%; N, 2.56%.

Calc. for $C_{23}H_{25}FeNRhCl$: C, 54.26%; H, 4.95%; N, 2.75%.

m.p. Melts from 159-163℃.

Attempted preparation of (1,5-cyclooctadiene)(2-ferrocenylpyridine)rhodium(I) chloride

μ-Chloro(1,5-cyclooctadiene)rhodium(I) dimer (0.025 g, 0.1 mmol monomer) and 2-ferrocenylpyridine (0.031 g, 0.1 mmol) were dissolved in dichloromethane (20 ml) and the mixture stirred for 50 min. The volume of dichloromethane was then reduced to approximately 5 ml under vacuum and excess diethyl ether added. The resulting precipitate was collected by filtration and washed with diethyl ether. Bright red crystals were recovered (0.017 g, 0.03 mmol, 33 %).

Properties:

Bright red crystals at room temperature. Air stable.

Characterisation:

^{1}H NMR ($CDCl_3$, 400 MHz) δ: too many signals for clear peak-picking.

Found:	C, 53.19%; H, 5.27%; N, 2.55%.
Calc. for $C_{23}H_{25}FeNRhCl$:	C, 54.26%; H, 4.95%; N, 2.75%.

m.p. Decomposes above approximately 130℃. Melts from 161-168℃.

*Attempted preparation of chlorobis(cyclooctene)rhodium(I)dimer (**26**)*

This was prepared according to a literature method (193). Rhodium trichloride trihydrate (2.153 g, 8.176 mmol) was dissolved in a 40:10 mix of isopropanol and dH2O (50 ml), previously deoxygenated by bubbling nitrogen gas. To this was added cyclooctene (6.00 ml, 5.08 g, 46.06 mmol). The mixture was stirred under nitrogen for 15 min, then sealed and allowed to stir for a further 7 days at room temperature. The resulting solid was collected by vacuum filtration, washed with ethanol, and dried under vacuum. The product (2.5 g, 6.97 mmol monomer, 85 %) was stored at 5℃ under nitrogen.

Properties:

Bright yellow/orange powder.

Characterisation:

^{1}H NMR Insoluble in all suitable solvents.

Found:	C, 52.48%; H, 7.03%.
Calc. for $C_{32}H_{52}Rh_2Cl_2$:	C, 53.56%; H, 7.81%.

m.p. Decomposes above approximately 130℃.

Attempted preparation of chloro(cyclooctene)(pyridine)rhodium(I)

This was prepared according to a modification of the method suggested by Dorta *et al* (194,195). (Chlorobis(cyclooctene)rhodium(I) (0.1956 g, 0.545 mmol monomer) was suspended in toluene (10 ml). To this was added pyridine (0.07 ml, 0.069 g, 0.87 mmol). The mixture is stirred for 270 min, after which the solid is collected by filtration and washed with diethyl ether. (0.1985 g, 0.453 mmol, 83 %)

Properties:

Deep orange powder. Stable in air.

Characterisation:

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.90 (br s), 7.53 (m), 5.31 (m), 2.16 (s), 1.76 (br s).

Found:	C, 46.60%; H, 4.17%; N, 4.27%.
Calc. for $C_{21}H_{33}NRhCl$:	C, 57.59%; H, 7.61%; N, 3.20%.

Attempted preparation of chloro(cyclooctene)(4-phenylpyridine)rhodium(I)

Chlorobis(cyclooctene)rhodium(I) (0.240 g, 0.669 mmol monomer) was suspended in toluene (10 ml). To this was added 4-phenylpyridine (0.1484 g, 0.96 mmol) in toluene (5 ml). The mixture was stirred for 255 min, after which the solid was collected by filtration, washed with toluene, and then with diethyl ether. (0.147 g, 0.285 mmol, 43 %)

Properties:

Pale brown powder. Stable in air.

Characterisation:

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 9.02 (s), 8.82 (s), 7.79 (s), 7.42 (m), 5.64 (m), 2.16 (br s), 1.63 (br s).

Found:	C, 33.39%; H, 3.95%; N, 5.69%.
Calc. for $C_{27}H_{37}NRhCl$:	C, 63.09%; H, 7.27%; N, 2.73%.

Chapter 3. Cytotoxicity and mechanism of action of precious metal complexes

Results and Discussion

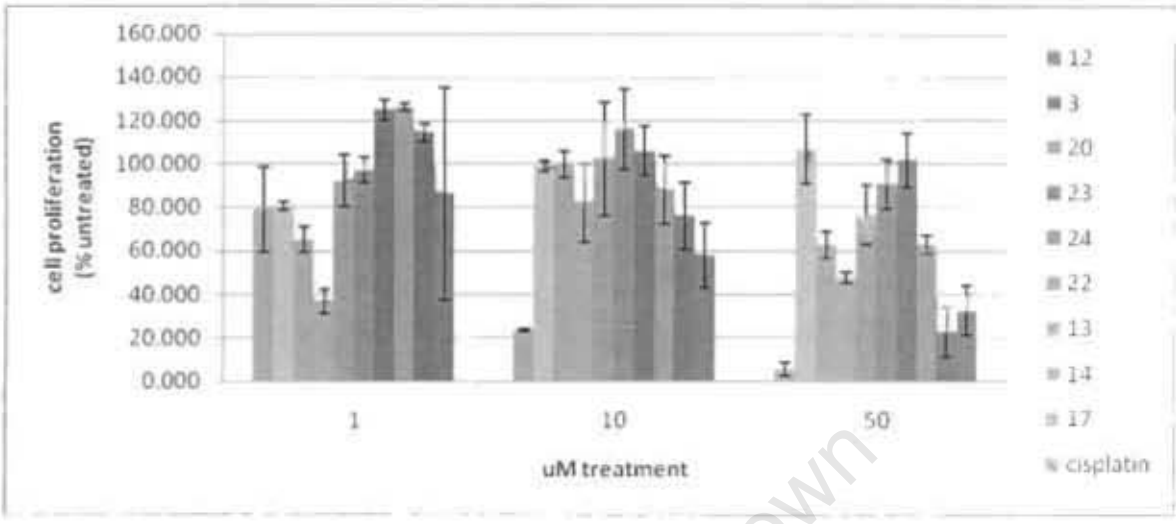
Preliminary screening

Screening assay

Preliminary screening of precious metal-containing compounds was carried out against WCHO1 cells, using the crystal violet assay, described below. The crystal violet test provides a rapid measure of cell proliferation by staining proteins in cells adhering to the culture dish. A limitation of this assay is that dead cells that remain attached to the culture dish, and other debris, may also yield positive results. Conversely, this assay will fail to detect still-living cells that have lifted from the dish, as occurs during mitosis. The other test used to determine cell proliferation was the MTT assay, wherein the staining compound produces a quantifiable colour change when metabolised in the mitochondria of living cells. This gives a more accurate estimate of cell number than the crystal violet assay, assuming that mitochondrial quantity and function remain reasonably consistent between cells under different treatment regimes. The less accurate crystal violet assay is used here with the sole intention of identifying which of the panel of compounds warranted special investigation.

The compounds tested included one gold(I) starting material (**3**); one gold(III) starting material (**12**); three phenylpyridine derivatives of gold(III) (**13**, **14**, **17**); one rhodium(I) starting material (**20**); three phenylpyridine derivatives of rhodium(I) (**22**, **23**, **24**); and cisplatin, as a positive control. Phenylpyridine complexes of gold(I) chloride were excluded because of their noted instability (particularly in DMSO, the solvent in which most treatment solutions are prepared. See the discussion above for more detail.) Of the compounds tested,

A



B

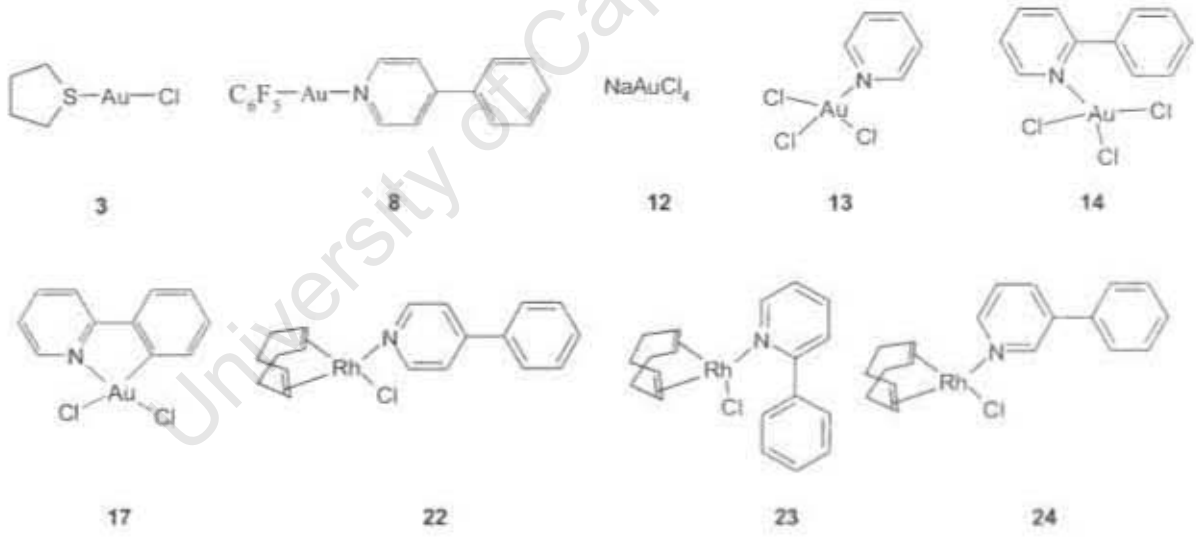


Figure 35. A: Preliminary screening of several metal-containing compounds, determined using the crystal violet assay. Each data point represents the result of 3 replicates, with the error bars indicating standard deviation. **B:** A table of the compounds tested in this assay.

it was found that only two displayed significant anticancer activity: **3**, and **12**, in addition to the positive control cisplatin. This result was disappointing, since the two compounds are well-known starting materials that have already been thoroughly characterised.

Evidence for the decomposition of rhodium(I) compounds

It is interesting to note that none of the rhodium-based complexes (**20**, **22**, **23**, **24**) displayed any significant activity. **22** had already been described as cytotoxic in earlier studies in our research group (88) and the other compounds are also reasonably similar analogues of this complex, thus making it surprising that no activity was observed. One consideration that may account for the observed discrepancy is that the results depicted above correspond to compounds dissolved in DMSO immediately prior to use; whereas these earlier studies were carried out using stock solutions of compound in DMSO, which may have been stored for some days prior to use. From this, we may hypothesise that the discrepancy is due to decomposition of these compounds in DMSO to yield more biologically potent species. Therefore, although the crystal violet experiment yielding these results was performed only once, it was decided to prioritise testing this hypothesis, via a comparison of the activity of freshly-prepared solutions with those of solutions that had been kept at room temperature for approximately 14 days. This was performed using the more reliable MTT EC_{50} assay.

From Figure 36, A-B, it should be clear that the gold(I) compound **3** decreases in activity after being left in DMSO, as naive expectation would predict. This is presumably associated with some decomposition reactions and, indeed, a precipitate is observed to come out of solution on a time scale of the order of days. In contrast (Figure 36, C-D), freshly-prepared **22** gives an EC_{50} value too high to be readily calculated from the standard concentration range of

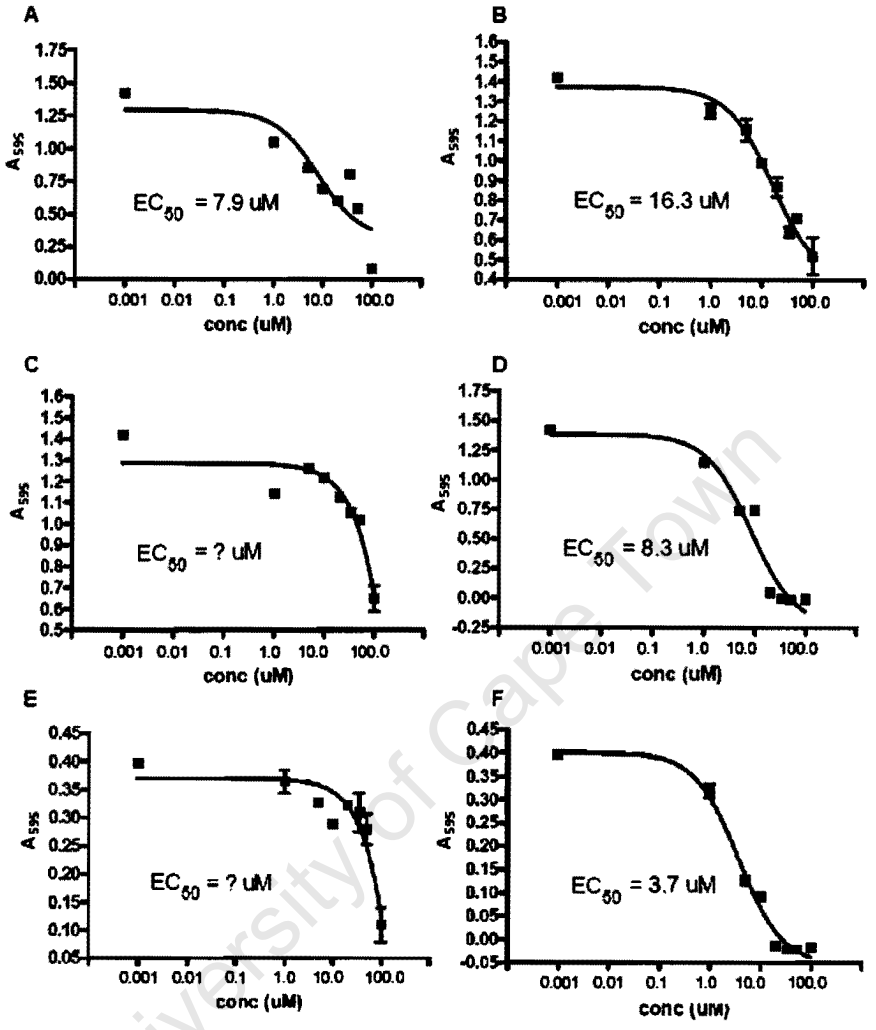


Figure 36. Comparison of cytotoxic activity between a gold(I) compound (**3**) and a rhodium(I) compound (**22**). Each compound has either been freshly made up in DMSO (**A**, compound **3**; **C** and **E**, compound **22**) or been allowed to stand in DMSO at room temperature (**B**, compound 3; **D** and **F**, compound **22**). The vertical axis of each plot depicts an absorbance reading directly proportional to cell viability. Vertical error bars represent the standard deviation due to variation between replicates of each data point. The horizontal axis gives concentration in µM, depicted logarithmically. **A** – **D** depict the results for the WHCO1 (oesophageal cancer) cell line; and **E** – **F** show results from the EPC2 (normal oesophageal tissue) cell line.

treatments used (1-100 μM), while "old" **22** gives a EC_{50} value 8.3 μM, about half that determined for cisplatin under the same conditions (15.0 μM). This seems to confirm the hypothesis that the cytotoxicity attributed to **22** in earlier studies is, in fact, due to its breakdown product in DMSO. It should also be noted that "old" **22** gives reasonably high cytotoxicity in both the cancer cell line (WHCO1) and the relatively normal cell line (EPC2, Figure 36, E-F), which does not lead one to infer that the compound kills cancer cells selectively. Nevertheless, it was decided that the breakdown product or other chemical species responsible for this cytotoxic activity should be identified.

Isolation of active rhodium(I) compound

Characterisation of decomposition process

This increase in activity of **22** over time is possibly associated with the displacement of one or other of the ligands on the complex with a DMSO molecule. This is plausible, given the reactions of DMSO with this type of rhodium centre observed in the literature (see Figure 34).

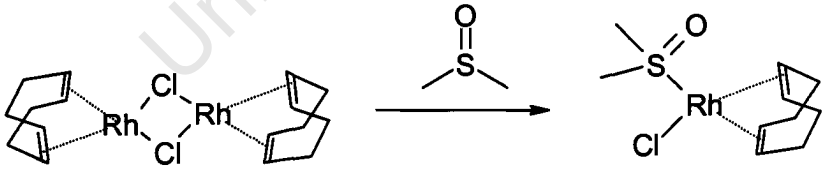
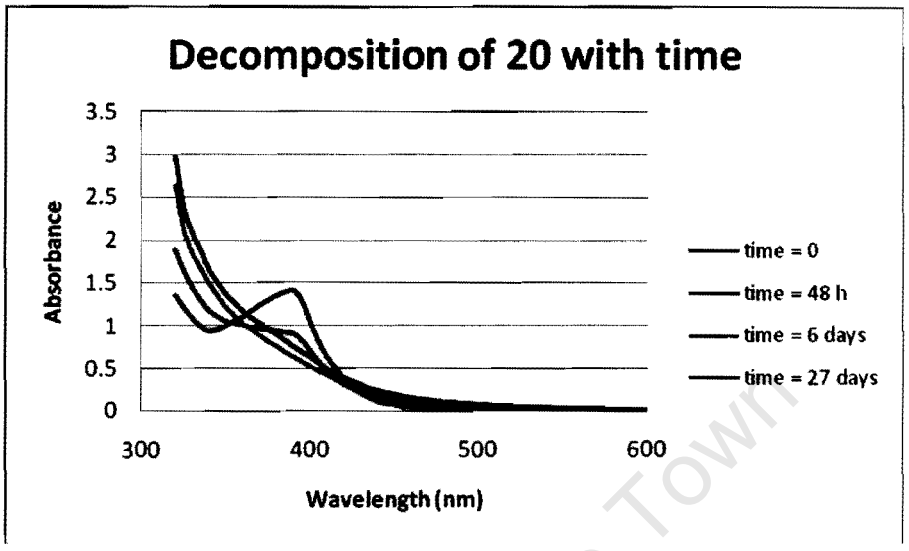


Figure 37. Reaction of DMSO with chloro(1,5-cyclooctadiene)rhodium(I) dimer, compound **20** (214)

Indeed, a solution of **22** in DMSO is observed to change from bright yellow to deep orange over the course of several days. Since electronic transitions in the rhodium(I) metal centre are responsible for the distinctive colours of these compounds, a change in colour would seem to indicate that some chemical reaction has taken place involving the metal centre. This process was monitored more rigorously by UV-Visible spectroscopy, depicted in Figure 38. From these results, several points are clear. Firstly, both **20** and **22** have characteristic peaks at 390 nm. The peak intensity for the dimer is exactly double that for **22** which, since the former has two moles of rhodium for each mole of compound, confirms that this peak is primarily due to the metal centre. Secondly, decomposition qualitatively appears to be associated with a decline in the intensity of this peak. Thirdly, it appears that the decomposition process is substantially complete after six days, since there is no substantial difference between the spectrum here and that some considerable time later.

A



B

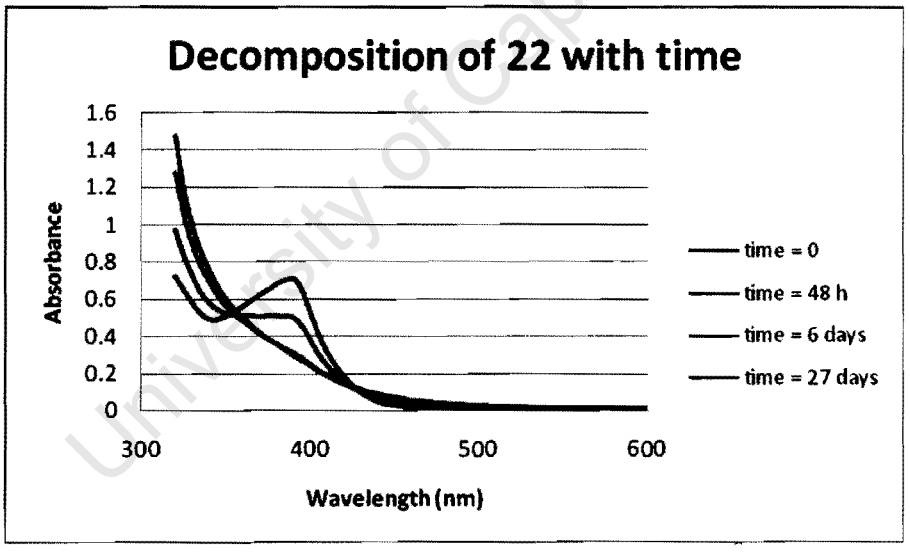


Figure 38. Decomposition of starting material **20** (**A**) and phenylpyridine-containing product **22** (**B**) dimer, monitored via UV-visible spectroscopy

This decomposition may also be monitored *in situ* via NMR in deuterated DMSO. This is depicted in Figure 39. Over time, in the aromatic region, new

peaks are observed to appear 8.49, 8.03, 7.98, and 7.91 ppm. The multiplet in the 7.50 ppm region also changes substantially. As these new peaks increase in magnitude, those peaks corresponding to the starting material decrease, indicating the interconversion of chemical species. At the latest time point, smaller sets of peaks centred on 8.82 and 8.64 ppm also appear.

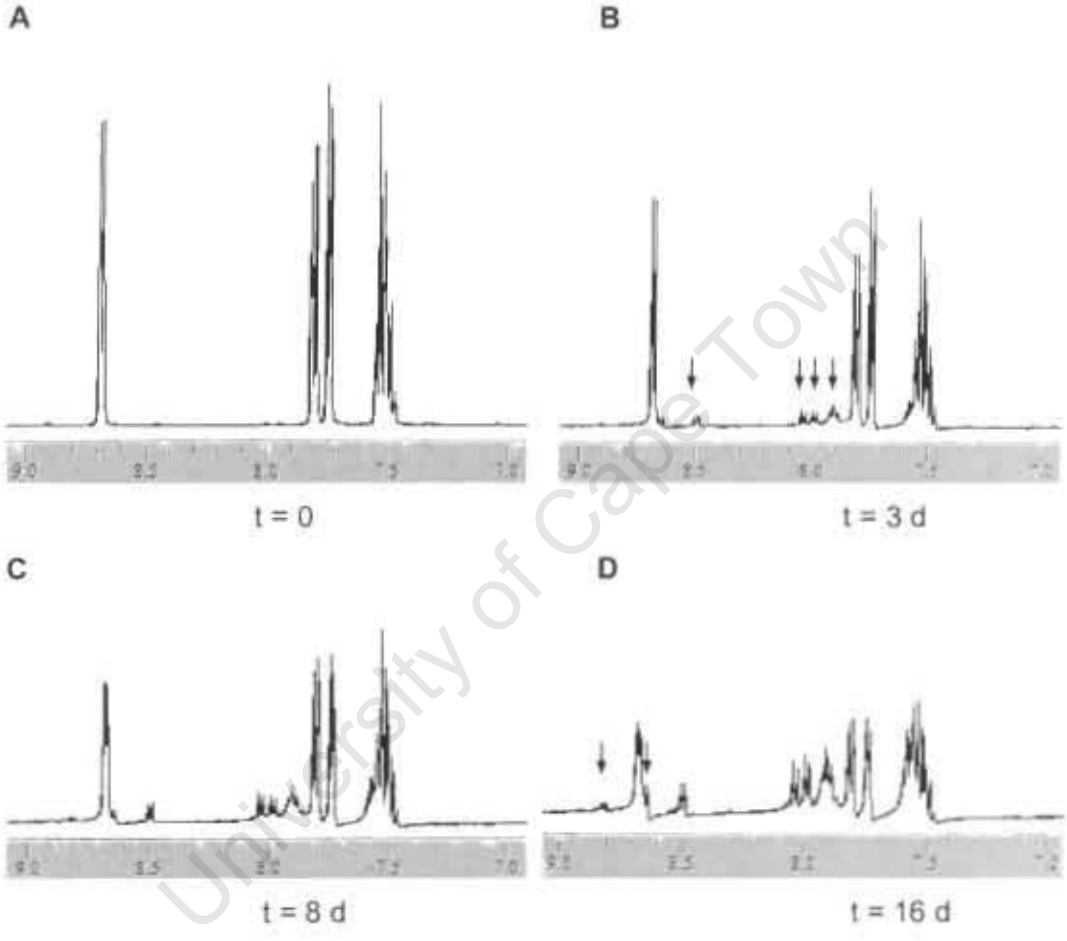


Figure 39. Decomposition of **22** in deuterated DMSO, carried out *in situ* in an NMR tube and monitored via ^{1}H NMR. New peaks are marked by arrows.

One very noticeable discrepancy between these data and the results for the UV-visible experiment is that chemical changes appear to take place on a much longer time scale. For instance, whereas the UV-visible spectroscopy appears to suggest that reactions are substantially complete by day 6, NMR spectroscopy

seems to indicate that distinct changes in the chemical environment of the aromatic ligand are still occurring at the end of the experiment on day 16. Several factors may account for this discrepancy. Firstly, the NMR experiment was carried out in a super-saturated solution of **22** in deuterated DMSO. This done in the hope that a larger quantity of **22** available for reaction with DMSO would result observable changes in the residual signals associated standard protonated DMSO. (As it happens, no such changes were observed, and the relevant region is not shown in Figure 39.) This means that unreacted solid **22** remained in the bottom of the tube in which the experiment was conducted, providing a continuous source of "fresh" compound. Secondly, it is possible that, because UV-visible spectroscopy records the total or resultant colour changes in solution, naive use of this technique may give an inaccurate picture of a system where several chemical reactions are occurring simultaneously. The colour changes observed in Figure 38 might, for instance, only correspond to the formation of the species associated with peaks at 8.49, 8.03, 7.98, and 7.91 ppm. Finally, it should be noted that the deuterated forms of various compounds are known to react slightly more slowly than their protonated equivalents, although this effect is small and unlikely to have a substantial impact here.

Isolation of breakdown products

The breakdown products of the decomposition of **22** were separated by means of partition across an aqueous/organic interface. A solution of the product in DMSO that had been allowed some time to decompose was mixed with a large excess of both toluene and water, and the various products allowed to partition into either phase over time. A substantial portion of the product was found to be soluble in neither phase, forming a flocculate on the phase interface. This material was collected to give the product **27**. ^{1}H NMR revealed the toluene fraction appeared to contain starting material (data not shown). The water fraction was not isolated, due to the usual difficulties of removing water as a

solvent. This represents something of a disappointment, as one would expect the water soluble fraction to be most mobile in physiological medium and thus most biologically active. Nevertheless, the isolated flocculate was characterised by NMR (Figure 40) and elemental analysis.

The ^{1}H NMR spectrum of **27** reveals some interesting features. It possesses non-solvent peaks that do not match any seen in the starting material (marked with arrows in Figure 40). One notable absence is any peak corresponding to 1,5-cyclooctadiene (expected at 4.12, 2.46 or 1.79 ppm), indicating that this ligand has been totally displaced from this product. Peaks corresponding to the N-donor ligand 4-phenylpyridine are present (at 8.82, 7.65, 7.49 ppm), indicating that this ligand has not been displaced. Each of this peaks has been somewhat shifted, however, demonstrating that the chemical environment in this decomposition product is different to that of compound **22**. The signal at 8.61 ppm is not obviously related to any seen for **22**, but may be a rhodium satellite peak. Particularly remarkable is the appearance of a very broad peak at 3.30 ppm, which is characteristic of DMSO bound to a metal centre via sulfur (195). However, if we compare this spectrum with that obtained for the *in situ* NMR experiment (Figure 39, above), we must conclude that **27** is *not* the major breakdown product. The aromatic peaks at 8.82 and 8.61ppm appear to correspond to the smaller peaks that appear only late in the decomposition process. The early decomposition products corresponding to the peaks at 8.49, 8.03, 7.98, and 7.91 ppm are not visible. The DMSO peak observed in Figure 40 is not evident as the experiment is conducted in deuterated DMSO, meaning any change in the chemical environment of DMSO will not be visible.

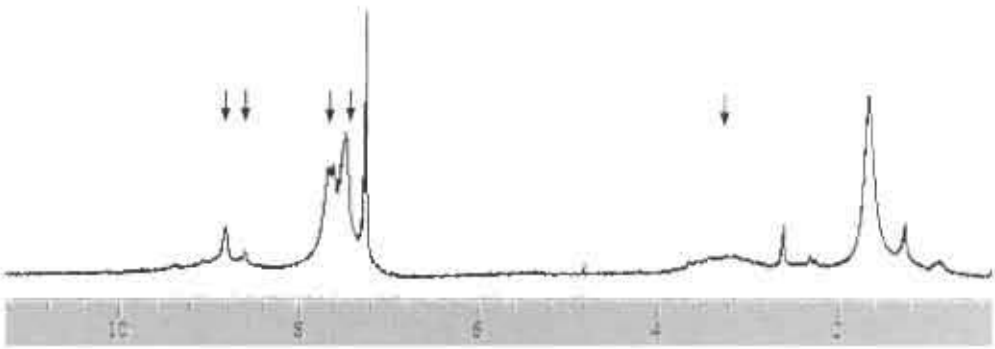


Figure 40. ^{1}H NMR spectrum of **27**. Several peaks are evident in the aromatic region, as expected. The absence of signals in the alkene region indicates that cyclooctadiene has been displaced; whereas the broad peak around 3.30 ppm is characteristic of DMSO coordinated via sulfur.

Dorta *et al* (215) have previously attempted the reaction of excess DMSO with μ-chloro(1,5-cyclooctadiene)rhodium(I) dimer, without success (Figure 41). However, the data from Figure 38 clearly suggest that some reaction does occur over time. Furthermore, these authors note that an NMR spectrum of the reaction solution (in deuterated toluene) reveals very broad signals for the alkene protons of 1,5-cyclooctadiene, indicating rapid and temporary binding of DMSO to the complex. The same authors (195,216) have prepared several compounds via reaction of excess DMSO with chlorobis(cyclooctene)rhodium(I) and its derivatives. In these experiments, DMSO tends to displace the cyclooctene ligands, resulting in a square planar complex with three DMSO and one chloride ligand. This suggests that the increased stability of chelating ligands compared to monodentate ones is responsible for the differences in reactivity.

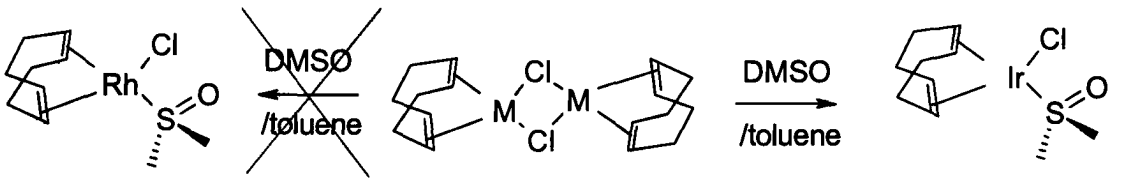
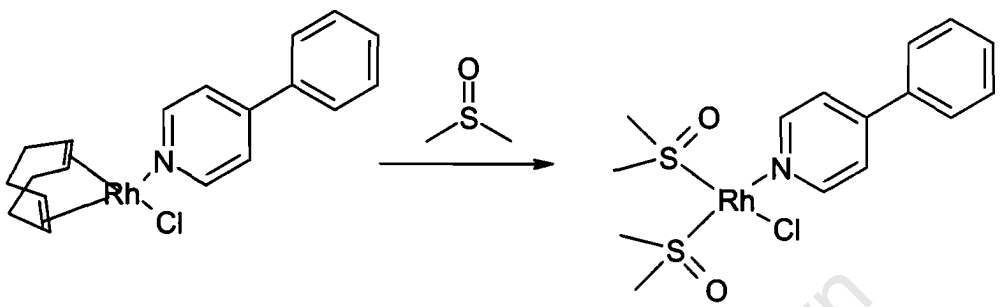


Figure 41. Attempted reaction of DMSO with rhodium(I) and iridium(I) chloro-bridged 1,5-cyclooctadiene dimmers (217)

Perhaps the greatly increased concentration (as opposed to simple stochiometric excess) of incoming ligand resulting from dissolution in DMSO allows some portion of dimer to be broken down into a DMSO-containing monomer as suggested by Figure 41. This process would be favoured by a shift in the reaction equilibrium due to the increased concentration, and by the very long reaction time. Unfortunately, in this work, the emphasis was on exploring those compounds with promising biological activity, and so characterisation data for the breakdown product of the dimer **20** have not been collected.

On the basis of these characterisation data and knowledge of the literature mentioned above, we might attempt to hypothesise a structure for **27**. Firstly, the NMR spectrum in Figure 40 implies that both 4-phenylpyridine and DMSO are present in the product and, as mentioned above, 1,5-cyclooctadiene appears to be absent. This indicates that the alkene groups around the metal centre have been replaced by DMSO, as Dorta *et al* (218) have observed with cyclooctadiene compounds. If we assume that this is the only structural change, we obtain the structure depicted in Figure 42. Elemental analysis confirms that both nitrogen and sulfur are present, confirming that the ligands mentioned above are found in the complex. However, the overall analysis does not entirely support the hypothesised structure, giving lower-than-predicted values for nitrogen (1.39 %, expecting 3.11 %) and sulfur (3.22 %, expecting 7.13 %)composition, and a slightly higher-than-expected value for carbon. This

suggests that some portion of cyclooctene-containing starting material (or reaction intermediate) might be present in the product.



27

Figure 42. Proposed structure for the breakdown product of compound **22** in rhodium

EC_{50} Determination

Creation of a cisplatin-resistant cell line

The WHCO1 cell line is commonly employed within our group for screening of potential anti-tumour compounds (219). In order to assess the degree of cross-resistance between cisplatin and the novel compounds described above, a cisplatin-resistant variant of WHCO1 cell line was created. A portion of these cells were exposed to prolonged low concentration cisplatin treatment (approximately a third of the EC_{50}, for several weeks), so as to produce a cisplatin-resistant population, denoted WCHO1-CR. The EC_{50} of both WHCO1-CR and the parent WHCO1 cell line with respect to cisplatin was determined (Figure 43). For the parent line, the EC_{50} was recorded as 15 μM (95% confidence interval: 8 – 29 μM); whereas that for the putative resistant line was 28 μM (95% confidence interval: 15 – 52 μM). This indicates that there is probably a real difference in cisplatin sensitivity between the cell lines.

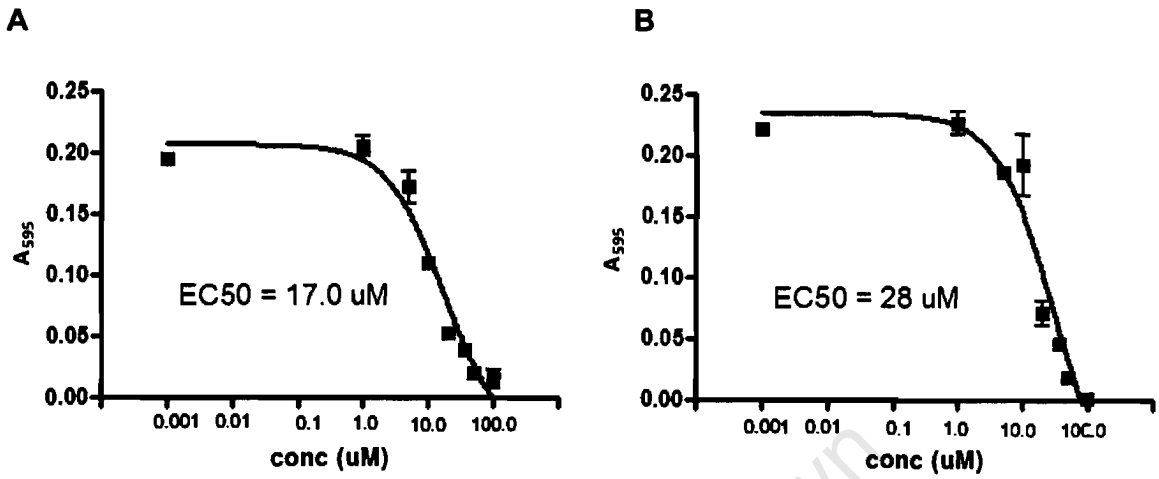


Figure 43. Comparison of cisplatin efficacy against parent WCHO1 cell line (A) versus putative resistant line (B). This figure is a representative sample from several replicates (see Table 1, below).

Characterisation of cytotoxic activity

The compounds selected for more rigorous EC_{50} screening were chosen according to several criteria. Firstly, the gold(I) chloride complexes were excluded because of their instability in water and DMSO, as discussed above. Secondly, starting materials were not included, on the grounds that they are well-known compounds, and so their properties are likely to be characterised already. Thirdly, all of the novel rhodium(I) complexes were eliminated, since **22** was felt to be an adequate representative for these, and because it is the best-characterised of them. It seems plausible that the decomposition products of these novel complexes display similar cytotoxic activity as that for **22**, given the similarity in their structures, and the fact that these also appear to change colour in DMSO solution. However, time constraints prevented the detailed testing of this hypothesis.

Cisplatin was included as a control, and to determine whether the putative cisplatin-resistant cell line did indeed display reduced sensitivity to this drug (see discussion above). Both freshly prepared and decomposed **22** were tested, to confirm the difference in activity between the two. The rhodium starting material **20**, both fresh and decomposed, was included to ascertain whether the activity observed for **22** was due simply to the metal centre, or whether the phenylpyridine ligand was also relevant to cytotoxic activity. **27** was screened to give an indication whether it did indeed represent the active component (or one of the active components) in the decomposition mixture of **22**. **14** was included as a representative of the gold(III) compounds. Since the stable pentafluorophenyl salts of gold(I) only became available after the completion of the screening assay described above, it was decided to include compound **8** as a representative of this family. Finally, 4-phenylpyridine was added to ensure that any activity observed for a given compound was due to the complex as a whole, not simply the organic ligand. Testing was carried out, in the first instance, against WCHO1, WHCO1-CR and EPC2.

The most important constraint on this experiment was that WHCO1 and EPC2 cells are only able to grow in different media. WHCO1 cells are normally cultured in serum-supplemented DMEM, which is an extremely protein-rich medium. In contrast, EPC2 cells are normally grown in minimally supplemented (i.e. serum-free) KSFM medium. This may mean there is a lower "effective concentration" of treatment compound in the serum-containing medium, because treatment compound tends to be sequestered by proteins in the serum and therefore not available to exert cytotoxic effect upon cells. Ideally, this effect could be eliminated by culturing the two cell lines in the same medium. Unfortunately, however, WHCO1 does not thrive in KSFM, nor does EPC2 in DMEM. As a compromise, it was decided to grow the WHCO1 cells in OptiMEM for the duration of the EC_{50} determination experiments. OptiMEM is a defined medium, containing far lower concentrations of growth factor and other proteins than supplemented medium. Unfortunately, the relative scarcity of growth factors

in OptiMEM results in WHCO1 cells growing very slowly in comparison to their normal behaviour (and, indeed, to the EPC2 cells). Since induction of cell death by cisplatin is, to a certain extent, dependent on cells attempting to progress rapidly through the cell cycle (see discussion of cisplatin mechanisms of action above), it is possible that the unusually slow-growing WHCO1 cells are disproportionately resistant to cisplatin under experimental conditions. Thus one confounding factor (the lower effective concentration of compound in supplemented medium) may have been swapped for another (the slower growth of WHCO1 in serum-free medium).

As noted earlier, and repeated here, those treatment compounds that were effective against WHCO1 were also effective against EPC2, meaning that they were not selective in targeting malignant cells more than normal cells (see Figure 44). As suggested above, however, this may be because WHCO1 cells are anomalously resistant to agents that target the cell cycle under low-serum conditions. Indeed, it should be noted that cisplatin also produced similar cytotoxic effects in both cell lines. Since cisplatin is known to be therapeutically effective, and to be selective in killing malignant cells at a greater rate than normal cells, the fact that this assay is unable to replicate this result may indicate that the test is not appropriate for estimating the selectivity of novel cytotoxic compounds. It was therefore decided not to continue using EPC2 cells as a treatment control.

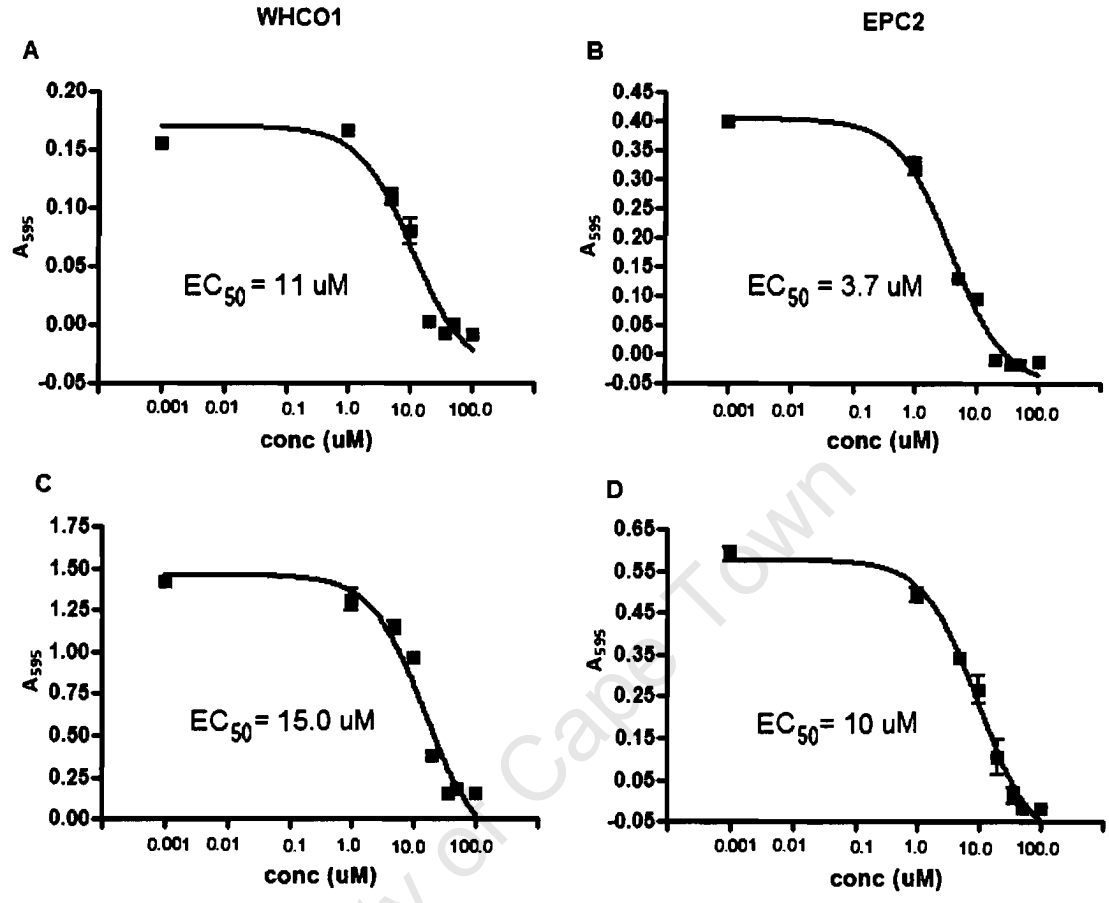


Figure 44. Cytotoxic effect of **22** (decomposed) (A and B) or cisplatin (C and D) on WHCO1 or EPC2 cells

As suggested earlier, it appears that both the impure decomposition product of **22** and the relatively pure **27** fraction exert cytotoxic activity (Figure 45, A and B). The EC_{50} value for each of these compounds was of the same order as that of cisplatin under the same conditions. Thus, despite its relatively small contribution to the overall composition of the breakdown product, **27** can nevertheless be regarded as an active component of the decomposition mixture. While, as seen above, fresh **22** does not exert significant cytotoxic activity, it is interesting that neither does either fresh or decomposed rhodium starting material (Figure 45, below). The software used to calculated EC_{50} values was

unable to do so for these data sets because complete cell death was not achieved, even at the highest test concentration used (100 μM). This implies that the biological activity is due not only to the interaction of DMSO with the metal centre (as is likely to be the case with any decomposition product isolated), but also to the pyridine-based ligand.

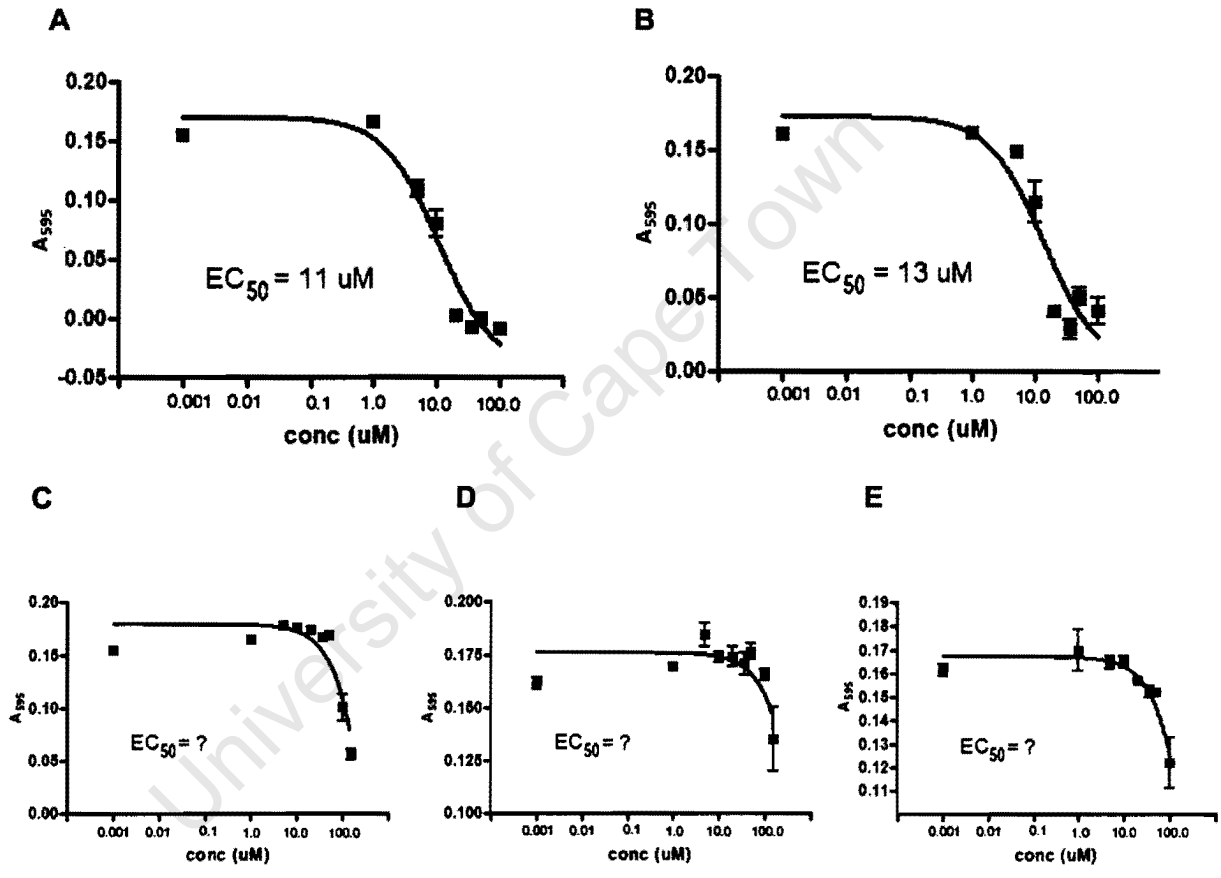


Figure 45. The cytotoxic effect of various rhodium compounds against WHCO1 cells. Decomposed **22** (A) has roughly the same activity as the purified form, **27** (B). None of fresh **22** (C); fresh (D) or decomposed (E) **20** shows any activity except at very high concentrations.

Another interesting observation is that WHCO1-CR displays greater resistance to the biologically active rhodium(I) compounds **22** and **27** than it does towards cisplatin (Figure 46), with significant cell death in evidence only at the highest treatment concentrations. If confirmed, this result implies that there is significant cross-resistance between cisplatin and the rhodium compounds, either because they induce cell death by the similar mechanism, or because they accumulate in the cytoplasm via the same processes.

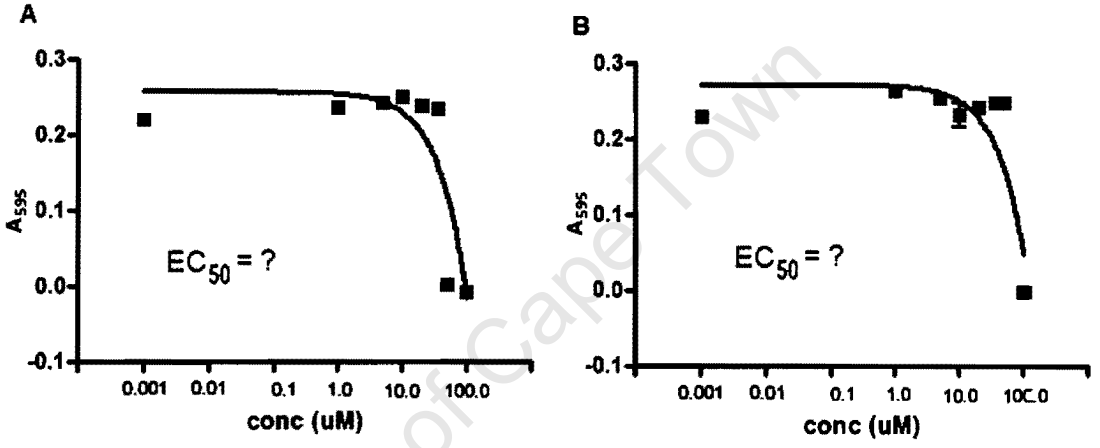


Figure 46 Biological activity of rhodium(I) breakdown products **22** (decomposed) (A) and the purified form, **27** (B) against WHCO1-CR. Neither displays any cytotoxic activity, except at very high concentrations.

Neither the gold(III) compound 14, nor the ligand 4-phenylpyridine by itself, display any significant cytotoxic activity, in any of the cell lines tested (Figure 47). This confirms the result from preliminary screening that the gold(III) complexes of the type under consideration do not appear to exert biological activity at reasonably attainable (i.e. low) concentrations. The measured absence of activity for 4-phenylpyridine confirms that the free ligand is not a significant contributor to the cytotoxicity of the pyridine-based complexes under discussion.

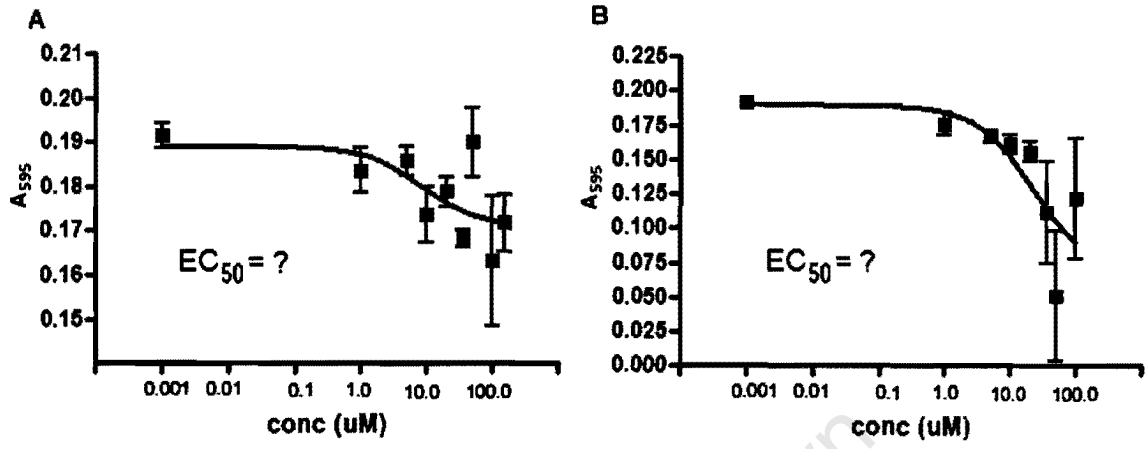


Figure 47. Cytotoxicity of **14** (A) and 4-phenylpyridine (B) against WHCO1 cells

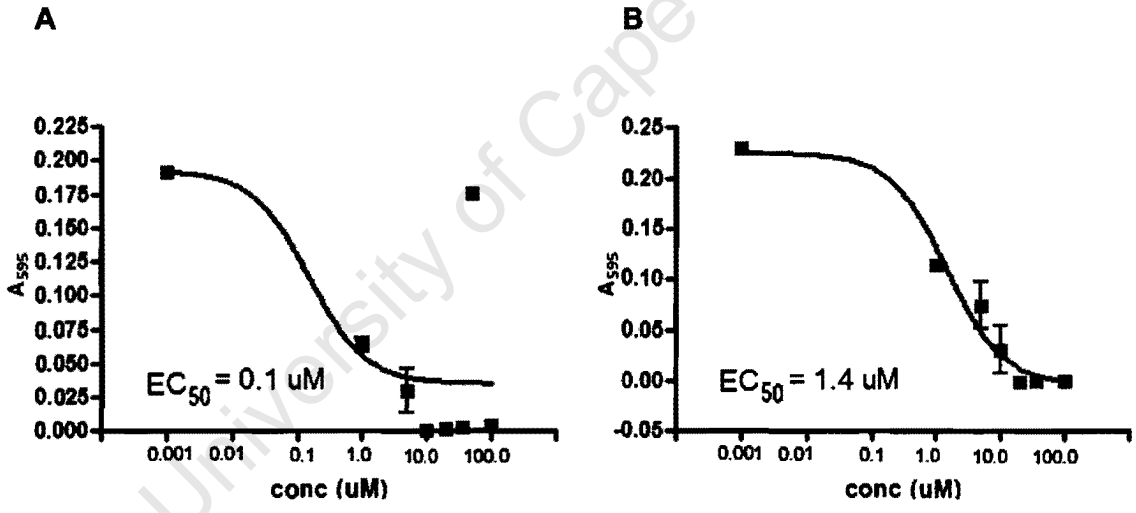


Figure 48. Biological activity of **8**, against WHCO1 (A) and WHCO1-CR (B) cell lines

The final result is rather unexpected, given the noted instability of the pyridine-based gold(I) complexes under discussion. **8** is found to exert remarkable toxicity against all cell lines under consideration (Figure 48). So robust was this

toxicity, in fact, that a statistically valid EC_{50} should not be inferred from the data presented in Figure 48, as cells are observed to be killed by all but the lowest concentrations of treatment compound. A repeat of this assay, using lower concentrations of **8**, is discussed below.

Repeated testing of promising compounds

On the basis of the experimental outcomes noted above, three compounds were deemed suitable for repeated testing, to ensure statistical validity of the stated results. In addition, cisplatin was tested further against WHCO1 and WHCO1-CR, in order to confirm the putative cisplatin resistance of the latter. **22** and **27** were included so as to define more clearly their respective cytotoxic activities relative to cisplatin and one another. As discussed above, the very potent activity of **8** stood in need of confirmation, specifically using lower concentrations to ensure better validity for the statistical calculation of EC_{50}. The results for these calculations are depicted in Table 1, below.

Table 1. Calculation of EC_{50} values for WHCO1 and WHCO1-CR cells with several promising compounds.

Cell line	Compound	Average EC50 (μM)	95% confidence interval (μM)	Replicates
WHCO1	Cisplatin	21	14 – 30	4
	22	12	8 – 17	3
	27	22	11 – 45	2
	8	0.3	0.1 – 0.6	2
WHCO1-CR	Cisplatin	31	26 – 36	2

detecting binding to DNA (221,222). Since the rhodium(I) centre of **22** is chemically similar to cisplatin, it might be expected also to interact with DNA, which would be detectable by similar means. Under the experimental protocol, a treatment compound is allowed to interact with plasmid DNA in solution, and any reaction is made evident by a noticeable shift in the mobility of the plasmid, due

Figure 49. An agarose gel showing purified plasmid extracts. Lane M1: a standard commercial marker where the successive bands are approximately 1.0 kilobases apart; lane M2: λ-phage DNA digested by the HindIII restriction enzyme. Molecular weights for the markers are shown down the sides of the figure. Lanes 1-5: individual plasmid preparations. The original colours of the gel picture have been inverted for easier viewing.

Several conclusions may be drawn from these data. Firstly, it seems that there remains a statistically significant difference in cisplatin cytotoxicity between WHCO1 and the putatively cisplatin resistant line WHCO1-CR. Several assays using 22 or 27 were performed against WHCO1-CR, but yielded plots similar to those depicted in Figure 46 and for which no numerical EC_{50} could be calculated. These experiments were therefore not cited in Table 1, but it should be noted that the earlier suggestion that these cells have developed cross-resistance against these compounds appears thus to be confirmed. Secondly, it appears that both **22** and **27** do display cytotoxic activity comparable to that of cisplatin under these experimental conditions. **27** appears to be less active, although this observation should not be considered reliable until the experiment has been repeated on a greater number of occasions and the statistical uncertainties thereby reduced.

Thirdly, it appears that **8** does, indeed, display quite considerable cytotoxicity, with an EC_{50} two orders of magnitude lower than that for cisplatin. In addition to this being a novel compound, it is also the first pentafluorophenyl-containing compound of gold(I) reported to possess cytotoxic activity. It is also one of very few gold(I) compounds containing a pyridine-based moiety reported to be biologically active, although (pyridine)gold(I) chloride has also been characterised (220).

DNA Binding assays

Gel-shift assay

From the screening assays above, it is known that **22** (after some decomposition in DMSO) is active against cancer cells *in vitro*. It was decided to explore its mechanism of action more fully. One of the common techniques for elucidating the mechanism of action of anticancer drugs is a gel mobility shift assay for

to topological changes induced by the chemical interaction. A readily available plasmid was chosen for this purpose – the pcDNA3.1 vector from Invitrogen – and purified by standard techniques (see the experimental section of this chapter). The results of this purification are shown in Figure 49.

The pure plasmid corresponding to lane 2 in Figure 49 was chosen for mobility studies. To perform these studies, pure plasmid was incubated at 37℃ in the presence of various treatment agents. As seen in Figure 50, plasmid incubated with the positive control, cisplatin, displays a very significant mobility shift, in line with results seen in the literature. The test compound, **22** produces a mobility shift of about the same magnitude as cisplatin, but only at double the concentration. Time course experiments reveal a pattern very much in line with expectations; with the degree of mobility shift roughly proportional to the time of incubation with treatment compound (see Figure 51). However the observed effects in these later experiments were extremely subtle compared to the result shown in Figure 50, raising the possibility that any observed mobility shifts may be attributable to slight deformations in the gel.

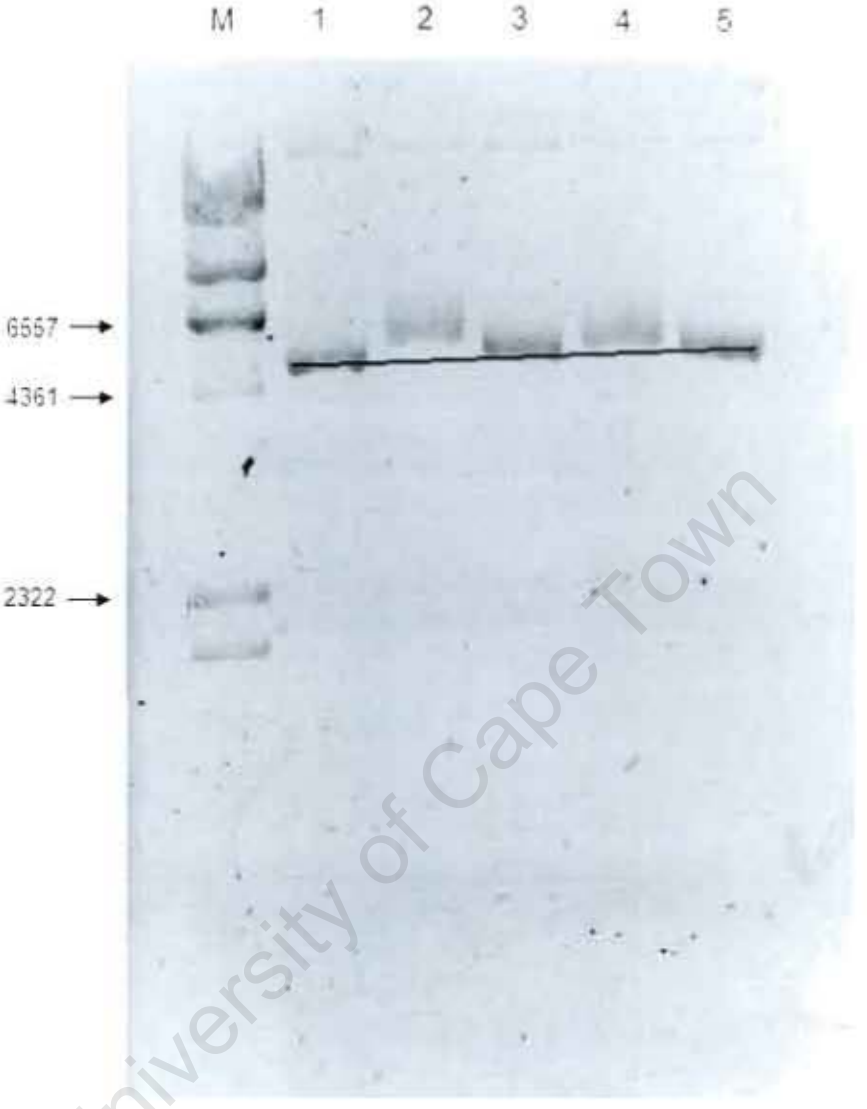


Figure 50. Gel mobility shift assay for interaction of compound **22** with plasmid DNA. Lane M: λ-phage DNA digested by the HindIII restriction enzyme; lanes 1 and 5: plasmid incubated with buffer only; lane 2: plasmid incubated in 11 nm cisplatin; lane 3: plasmid incubated in 11 nm compound **22**; lane 4: plasmid incubated in 22 nm compound **22**. Each sample was incubated for 1 hour at 37℃. A line has been drawn between bands corresponding to the supercoiled form of DNA in both control lanes. The original colours of the gel picture have been inverted for easier viewing.

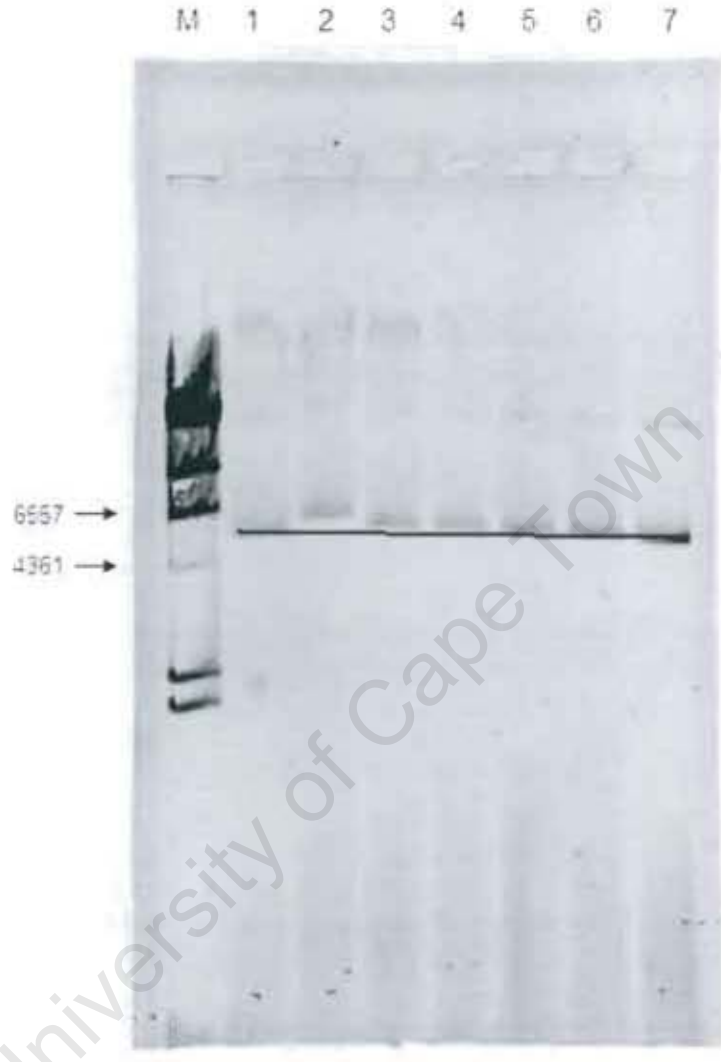


Figure 51. Mobility shift assay showing the interaction of **22** with plasmid DNA over time; a representative result from two repeats of the experiment. Lane M: λ-phage DNA digested by the HindIII restriction enzyme; lane 1: plasmid incubated with buffer only (3 hour incubation); lane 2: incubation with 11 nm cisplatin (3 hours); lane 3: incubation with 11 nm of **22** (3 hours); lane 4: incubation with 22 nm **22** (3 hours); lane 5: incubation with 22 nm **22** (2 hours); lane 6: incubation with 22 nm **22** (1 hour); lane 7: incubation with buffer only (1 hour).

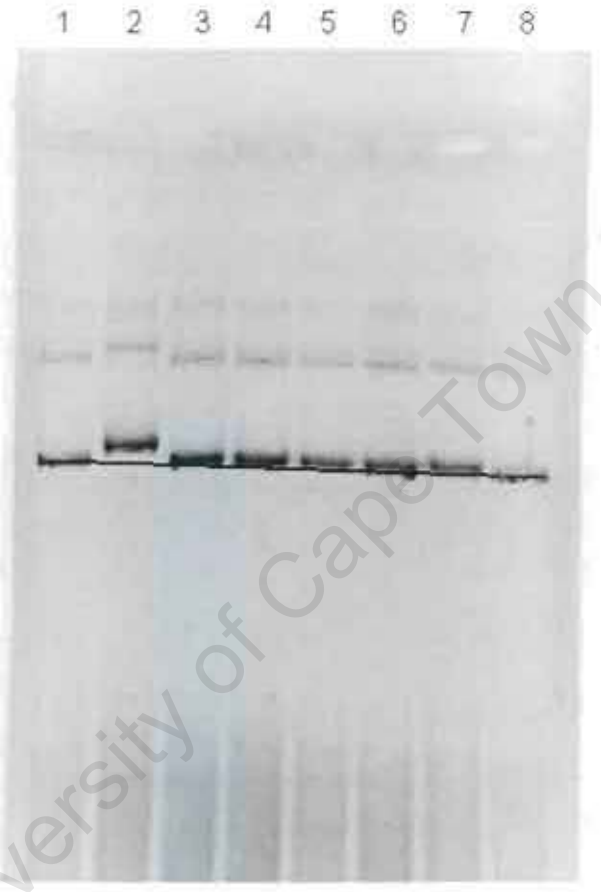


Figure 52. Mobility shift assay showing the interaction of various formulations of **22** with plasmid DNA. Lanes 1, 5 and 8: buffer only; lane 2: incubation with 11 nm cisplatin; lane 3: incubation with 11 nm **22**, using the same dilute DMSO stock used to carry out the incubations in the experiments described above (Figure 50 and Figure 51); lane 5: incubation with 11 nm **22**, using a dilute stock freshly made from powder (no decomposition); lane 6: incubation with dilute 11 nm **22**, using a dilute stock solution freshly made up from concentrated stock solution; lane 7: incubation with 110 nm **22**, using the concentrated stock used to prepare more dilute treatment solutions in earlier experiments.

As it is already known that **22** undergoes decomposition in DMSO, and that secondary decomposition products form after greater periods in solution (see Figure 39 and associated discussion), it may be speculated that the decline in performance of the treatment may have been due to further degradation of the compound. It was therefore decided to carry out similar experiments to those depicted above using various "batches" of treatment compound. The result of this is depicted in Figure 52. The result of this assay was, if anything, even less satisfactory than the time course experiments. Only treatment with ten times the normal concentration of compound yielded any noticeable gel shift, and even this may be interpreted as the consequence of gel distortions.

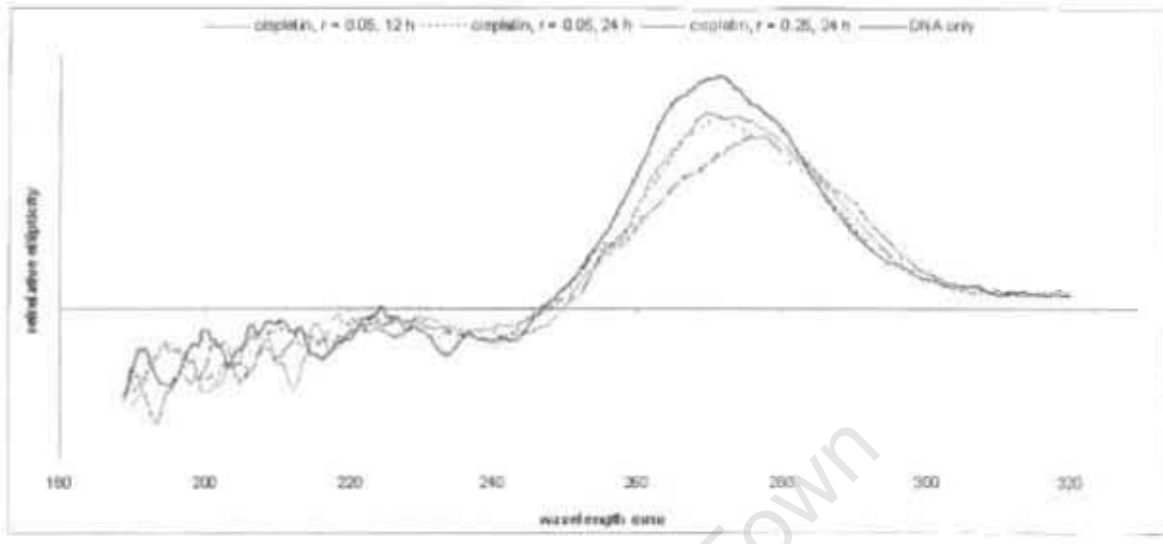
Circular dichroism assay

As reproducibility could not be achieved for the gel shift assay, despite the initially promising results, it was decided that a different DNA binding assay might give more consistent results. Another method employed commonly in the literature, including for the detection of cisplatin interaction with DNA, is circular dichroism (223). This is a form of spectroscopy which measures the relative absorption of left-handed versus right-handed circularly polarised light by a biomolecule (224,225). Since many biomolecules are chiral (i.e. have handedness), the relative absorption spectra may be quite different and so many of these molecules have distinctive circular dichroism signatures. In addition to the intrinsic handedness of biological monomers, large biomolecules such as proteins and DNA may also be chiral due to secondary structure. DNA is chiral due to the helical arrangement of the ribose sugars in its backbone. However, since these sugars typically have absorption peaks at wavelengths lower than 190 nm, which is about the limit of most available instrumentation, it is usually the absorption spectra due to the nitrogenous bases that are measured by circular dichroism. Changes in the chirality of the molecule as a whole, including

that caused by intercalation or chemical modification of bases, can be observed quite readily.

In the spectra shown in Figure 53, the characteristic spectrum of duplex DNA is clearly visible, including a "positive band" with a maximum at 283 nm. The spectrum of DNA usually also contains a broad "negative band" from 220-260 nm. However, this is not visible here because the DMSO used in the treatment buffer gives more intense signals in this region. Treatment with cisplatin at the concentrations applied was observed to give rise to a decrease in the intensity of this positive band (Figure 53, A). The degree of decrease in band intensity was greater for the larger concentration of cisplatin, as might be expected. However, no substantial difference was observed between 12 h and 24 h incubation, most likely due to saturation. Novel rhodium compounds **22** and **27** were also included in the same experiment (Figure 53, B). It should be clear that that treatment with these compounds gives rise to a decrease in band intensity, as with cisplatin. This serves to confirm the initial positive results of the gel shift experiment that could not be reproduced successfully. Again, there is no obvious difference in the spectra corresponding to different incubation times with the same compound, implying that the 12 h treatment is sufficient to achieve saturation. Both cisplatin and **22** give rise to similar alterations in band intensity at the r = 0.05 treatment concentration. At r = 0.25, both cisplatin and **22** give rise to a very pronounced decline in signal intensity, although that for **22** is more marked. However, this may be due to the fact that **22** was added to the sample in DMSO, and the blank controlled for only the amount of DMSO present in the r = 0.05 treatment concentration.

A



B

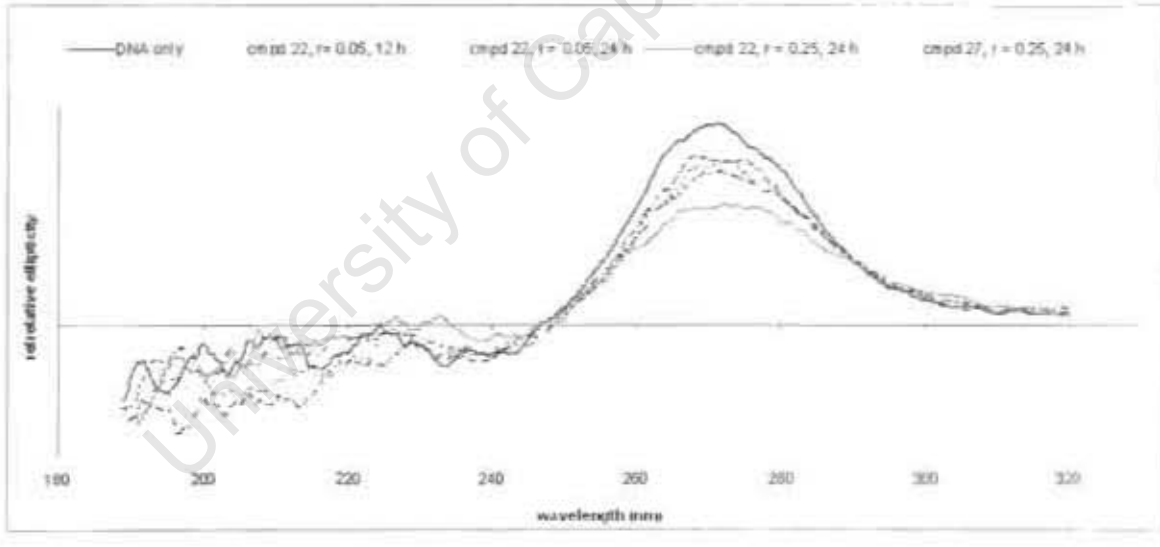


Figure 53. Circular dichroism measurement of cisplatin (**A**), **22** (decomposed) and **27** (**B**) interacting with pcDNA3.1 plasmid. The concentration and incubation time for each treatment is listed for each data set.

A decrease in the ellipticity of the positive band is commonly observed in response to metal treatment of DNA. This is often attributed to a transition from the B- to C-form of DNA (226-230), for several reasons. The C-conformation of DNA differs from the better-known B-form primarily in that it possesses a far less extensive hydration shell and is consequently somewhat compressed, with polymer length per base and the volume of the major groove reduced. The C-form is also more "tightly wound", in that the turn angle between successive bases is greater, meaning that there are fewer bases per turn of the double helix as a whole. To counter electrostatic repulsion between negatively charged phosphates in the DNA backbone, the C-conformation tends to retain more cations within its immediate chemical environment (231). The B→C transition usually occurs due to a reduction in water content of the DNA, a process that is enhanced in a solution with high salt concentration, particularly if the cation is lithium. (231). Two primary pieces of evidence suggest that this transition is occurring upon cisplatin binding. The first is the B→C transition is accompanied by a dramatic decline in circular dichroism peak intensity for the positive band of the affected DNA (see Figure 54). The second is that cisplatin treatment induces a reduction in polymer length that is measurable by electron microscopy (232). Another proposed mechanism for the observed decrease in ellipticity is localised denaturation – that is, partial unwinding of the double helix – caused by adduct formation (229,233). In contrast, increases in the ellipticity of the positive band are attributed to non-denaturing distortions in the DNA secondary structure, caused by the bivalent attachment of cisplatin to adjacent guanine bases (229). These sorts of adducts are known to cause the DNA double helix to bend considerably (234), which may eliminate the base-stacking interactions that give rise to the relevant absorption band.

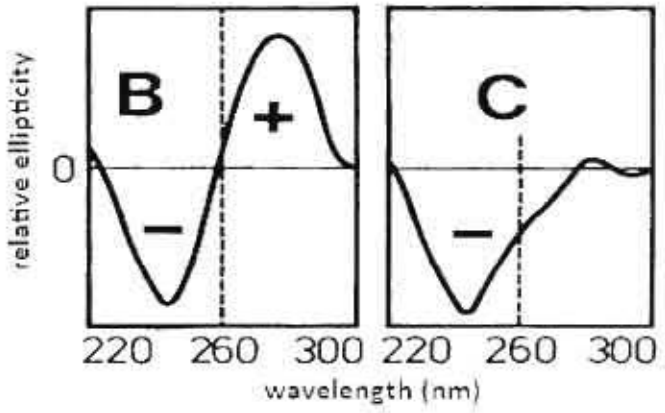


Figure 54. Circular dichroism spectra for the B- and C-forms of DNA. Adapted from (231)

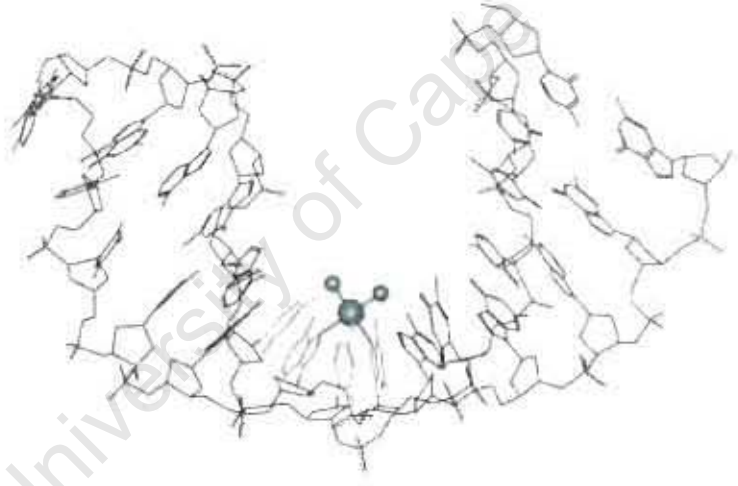


Figure 55. Crystal structure of a cisplatin adduct at dGpG, showing how the normal geometry is bent (235)

Most of the results above refer to experiments conducted on calf thymus DNA, in contrast to the plasmid DNA used in this work. This is not expected to make a large difference to the results, however, since the ultraviolet region to which circular dichroism is sensitive reflects mainly base-stacking behaviour, rather than the larger structure of the biomolecule (231). Macquet and Butour (236)

have carried out a reasonably comprehensive study of the effects of differing concentrations of cisplatin on genomic DNA from various types of bacterium, and salmon sperm, with consequently differing tertiary structure and base composition. In each case, they observed that increasing concentrations of cisplatin, to a maximum r-value of 0.1 or 0.125, producing increasing peak intensity in the positive band, followed by steadily decreasing intensity. For each sample, the minimum recorded intensity occurred at r = 0.4, with peak height distinctly less than that of unaltered DNA. (Akdi *et al* (237) note a very similar dose dependency on plasmid DNA, albeit using a palladium compound. It therefore seems plausible that the overall effect of the first few molecules of cisplatin to form adducts on the DNA molecule create localised distortions that distort nucleotide interactions in such a way as to increase the signal of the 280 nm peak. However, as greater numbers of adducts form, large-scale distortions of the biopolymer take place, giving rise to changes in the overall conformation and regions where the double helix is completely denatured.

Macquet and Butour (236) also note that increasing cisplatin concentration results in a steady shift of peak location to higher wavelengths. This latter effect can be observed with the r = 0.25 concentration of cisplatin (Figure 53, A); but is not observable in treatment with the rhodium compounds, despite comparable decreases in band intensity. This does suggest a different mode of binding between cisplatin and the rhodium compounds. A further question that may be asked is why the metal treatments depicted in Figure 53 bring about a uniform decrease in band intensity, despite the fact that we might expect *increased* intensity at lower concentrations of treatment. The difference may stem from the buffer conditions applied. Since, according to the standard protocol, the rhodium treatment compounds used in the experiments described above are dissolved in DMSO, DMSO was added to each of the treatment tubes requiring addition of these compounds. To maintain consistency, an equal quantity of DMSO (1% v/v) was added to each of the other test tubes as well. As described earlier, this had the effect of altering the lower wavelength end of the spectrum.

In addition, however, DMSO is known to promote denaturation of DNA by interfering with base pairing, and is commonly used for this purpose in promoting temporary denaturation in the polymerase chain reaction (238). This suggests that the experimental protocol inadvertently promoted denaturation, one of the processes by which the intensity of the characteristic positive band of B-form DNA is reduced in experiments of this type. Thus, although these results do confirm that these compounds do interact with DNA (recall that the spectra representing treated DNA still differed substantially from that denoting the DNA-only control, even though each contained equal amounts of DMSO), they do not give us sufficient information as to how this interaction may occur under *in vivo* conditions.

Experimental

Isolation of active rhodium(I) compound

Decomposition of (1,5-cyclooctadiene)(4-phenylpyridine)rhodium(I) chloride in DMSO

Compound **22** ((1,5-cyclooctadiene)(4-phenylpyridine)rhodium(I) chloride, 0.040 g, 0.100 mmol) was dissolved in neat DMSO (3 ml) to give a 33.3 mM solution. The solution was allowed to stand at room temperature for several days. Over time, the solution was observed to change in colour from yellow to a deep brick red. These colour changes were recorded using a Varian Cary UV-Visible Spectrometer, using pure DMSO as a blank.

This decomposition was also monitored by NMR, as follows. 1,5-cyclooctadiene)(4-phenylpyridine)rhodium(I) chloride (0.039 g, 0.098 mmol) was added to DMSO-d6 (0.978 ml) to form a supersaturated solution, with some

undissolved compound remaining at the bottom of the vessel. ^{1}H NMR spectra were recorded over the course of several days.

*Isolation of the breakdown product of (1,5-cyclooctadiene)(4-phenylpyridine)rhodium(I) chloride in DMSO (**27**)*

After the decomposition of (1,5-cyclooctadiene)(4-phenylpyridine)rhodium(I) chloride in DMSO was complete, as determined by UV-Visible spectroscopy, an aliquot of solution (2.5 ml of 33.3 mM solution) was added to toluene (20 ml) to produce a fine suspension. This mixture was added to H_2O (15 ml), and the biphasic mixture was thoroughly shaken in a separating funnel. A large amount of flocculate was observed to form on the interface between the two phases. This was separated from the toluene layer by successive washes with water. The flocculate was collected from the aqueous phase by filtration, and recovered from the filter by washing with dichloromethane. Solvent was then removed in vacuo to yield a deep red solid (0.004 g, 9 µmol, 11 %). Yield calculated for $C_{15}H_{21}NO_2S_2RhCl$.

Properties:

Fine, deep red powder.

Characterisation:

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.83 (br s, 2H), 7.65 (d, 2H), 7.48 (br s, 5H), 3.23 (br s)

Found:	C, 44.79; H, 4.72; N, 1.39; S, 3.22.
Calc. for $C_{15}H_{21}NO_2S_2RhCl$:	C, 40.05; H, 4.71; N, 3.11; S, 7.13.

m.p.: None recorded.

Cell culture

Culture conditions

Two cell lines were used. WHCO1 is derived from a biopsy of a South African patient with malignant squamous cell oesophageal carcinoma (239), and EPC2 is derived from healthy squamous epithelial cells of the oesophagus, which have been immortalised *via* transfection with the hTERT gene (240). All cells were cultured in a humidified atmosphere at 37℃ with 5% CO_2. WHCO1 cells were grown either in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% foetal bovine serum and with penicillin (100 U/ml) and streptomycin (100 µg/ml); or in OptiMEM (Invitrogen), without any additional supplementation, but with penicillin (100 U/ml) and streptomycin (100 µg/ml). These cells were normally grown in supplemented DMEM, and crystal violet assays were carried out in this medium. For the EC_{50} determinations shown above, WHCO1 cells were transferred into OptiMEM. EPC2 cells were grown in defined K-SFM (Gibco), supplemented with bovine pituitary extract (40 µg/ml), EGF (1.0 ng/ml), penicillin (100 U/ml) and streptomycin (100 µg/ml).

Passaging of cells

Both cell types were passaged as follows. Cells were covered with a minimal layer of trypsin solution. After cells began to visibly lift from the dish, they were gently suspended by repeated aspiration and expulsion of the trypsin solution. For cells cultured in OptiMEM or KSFM, an equal volume of soybean trypsin inhibitor solution (Sigma; 0.25 mg/ml) was added to neutralise the trypsin; whereas for cells cultured in supplement DMEM, an equal volume of culture medium was used for this purpose. The cells were harvested by centrifugation,

resuspended in culture medium, counted using a haemocytometer if necessary, and plated into a new culture dish.

Freezing and thawing of cells

To freeze, each cell line was grown to confluence in a 100mm culture dish. The cells were then trypsinised as described above, collected by centrifugation, resuspended in culture medium and counted using a haemocytometer. The cells were again collected by centrifugation and resuspended in freezing medium at a concentration of 1 million cells per millilitre. For WHCO1 cells, this freezing medium consisted of complete cell culture medium (90 % v/v) and DMSO (10 % volume). For EPC2 cells, freezing medium consisted of foetal bovine serum (90 % v/v) and DMSO (10 % volume). The cells in freezing medium were aliquotted into plastic cryogenic tubes (1 mL per tube) and then frozen in a -80℃ refrigerator. After freezing, cells were transferred to liquid nitrogen for storage.

To thaw cells, a tube was suspended in a 37℃ water bath for 1 minute. The freezing medium containing the cells was added to a test tube and diluted 9:1 with the culture medium appropriate for that cell line (although just-thawed WHCO1 cells did not grow successfully in OptiMEM). The cells were then collected by centrifugation, resuspended in culture medium and seeded into a culture dish.

Creation of cisplatin-resistant line

This was carried out along the same lines as previously reported (241). WHCO1 cells were cultured in a 60 mm dish in supplemented DMEM, in the presence of cisplatin (6.68 μM). The cells were monitored carefully for signs of cell death, growth arrest and/or abnormal morphology. After some time (usually two

passages of approximately three days each), the cells were judged to be in danger of permanent loss of viability. They were then transferred into cisplatin-free supplemented DMEM to "recover". The cells were now monitored for the return of normal morphology and growth, whereupon they were returned to cisplatin treatment (usually after one passage of approximately five days). The cells were thus faced with several stringent selection events for cisplatin. After five rounds of selection, these cells were maintained in the same way as unaltered WHCO1 cells for use in experiments.

Cytotoxicity screening

For each screening assay, cells were seeded in 90 µl culture medium at a density of 3000 cells in each well of a standard 96-well plate. WHCO1 cells were transferred from their normal supplemented DMEM growth medium into OptiMEM. Treatment compounds were made up in medium at the desired concentration and then added to the wells in 10 µl medium 24 h after seeding. The final concentration of DMSO never exceeded 0.4 % in treatment culture, and had no impact on cell growth at this concentration. The cells were allowed to grow in the presence of compound for 48 h.

The crystal violet assay was performed as follows. Medium was decanted from the 96-well plate and the cells fixed for 10 min in 100 % methanol. The methanol was poured off and 1 % w/v crystal violet solution in 1:1 methanol:water solution added. The cells were allowed to stain for 20 min, after which the staining solution was decanted and the cells rinsed with water. The crystal violet stain was solubilised in 1:1 glacial acetic acid:water. Plates were then read at 595 nm on a BioTek EL800 Instrument.

The MTT Cell Proliferation Kit (Roche) was used as per the manufacturer's instructions. Briefly, 10 µl of MTT (10 mg/ml in PBS) was added to cells after treatment. The reaction was allowed to proceed for 4 h and 100 µl of solubilisation buffer (10 % SDS in 0.01 M HCl) added. After overnight solubilisation at 37℃, the plates were read at 595 nm. All data points were carried out in triplicate. Data were analysed and EC_{50} values calculated using Prism 4.03 software (Graphpad Software, Inc). The EC_{50} value refers to the concentration of compound required to produce a reduction in cell number of 50 %. Average values and confidence intervals EC_{50} were calculated with Microsoft Excel, using a modification of the statistical technique suggested by the manufacturers of the Prism software (242).

DNA Binding Assays

Plasmid DNA extraction

Plasmid DNA was extracted from a culture of XL1-Bluescript strain of *Escherichia coli* containing the pcDNA3.1 plasmid (Invitrogen), the starter culture being kindly provided by Caryn Buchanan.

Briefly, a bacterial culture was grown overnight (OD_{600} reaching 1.3) in Luria Broth (1 % w/v tryptone, 0.5 % w/v yeast extract, 0.5 % w/v NaCl, 1 mM NaOH). Cells were harvested from 1.5 ml of each culture by centrifugation (4000 rpm, 5 min). DNA was then extracted by one of two ways. The Peqlab E.Z.N.A.® Plasmid Miniprep Kit (Peqlab Biotechnologie GmbH, Erlangen, Germany) was used, as per the manufacturer's instructions. Alternatively, the cells were resuspended in 0.2 ml resuspension buffer (1 % w/v glucose in TE (25 mM Tris-HCl, 50 mM EDTA, pH 8.0)). 0.4 ml of denaturation buffer (0.2 M NaOH, 25 % w/v SDS) was added and the solution placed on ice for 5 min. 0.3 ml of precipitation buffer (11.5 % v/v glacial acetic acid, 3 M potassium acetate) was

added to each sample. The resulting precipitate of cellular debris was removed by centrifugation (12000 rpm, 10 min) and the supernatant recovered. Isopropanol (0.6 volumes) was then added to each sample, which was mixed and left for 10 min at room temperature. DNA was collected by centrifugation (12000 rpm, 10 min) and the supernatant discarded. The pellet was washed in 80 % ethanol and recollected by centrifugation. The supernatant was discarded and the pellet air-dried. DNA from each sample was resuspended in either TE or distilled water (0.2 ml).

Agarose gel mobility shift assay

The DNA binding assay uses the method of Lippard *et al* (243). To prepare a sample for a DNA binding assay, DNA (1µl, 0.3 µg) was placed in an eppendorf tube. To this was added dH_2O (3 µl), followed by treatment compound diluted to the appropriate concentration in dH_2O (1 µl). The reaction volume was collected in the bottom of the tube by brief centrifugation, and the tube incubated in a water bath at 37℃ for the appropriate amount of time. Each tube was centrifuged briefly at hourly intervals to ensure liquid remained concentrated in the bottom of the tube. After the incubation period was complete, loading buffer (1 µl, 30 % glycerol in saturated bromophenol blue solution) was added to each tube

The DNA solutions were electrophoresed on a 1 % agarose gel at 100 V (~7 V/cm) for 105 min, using TBE buffer (90 mM Tris, 90 mM boric acid, 2.2 mM EDTA, pH 8.4). After electrophoresis, the gel was stained in ethidium bromide (0.001 % w/v), rinsed in dH_2O with shaking, and visualised under ultraviolet light.

Circular dichroism experiment

Plasmid DNA in distilled water was added to further distilled water to make up volume for spectrometry, with final DNA concentration in the range 0.05 - 0.2 µg/µgl. To this was added either cisplatin in 0.15 M saline (1.67 mM solution, Pharmachemie (Pty) Ltd) or rhodium-containing test compound in DMSO (3.3 mM solution). The molar concentration of DNA bases was calculated using 330 g/mol bases, and the desired amount of treatment compound added accordingly. The overall concentration of both NaCl and DMSO was kept constant across all experimental samples. Each sample was incubated at 37℃ for the desired time period, and analysed by circular dichroism at this temperature. Spectrometry was carried out using a Jasco 810 spectropolarimeter (Jasco, Easton, MD).

Chapter 4. Conclusion and Future Work

Chemistry

In this work, novel pyridine-based complexes of gold(I), gold(III) and rhodium(I) have been prepared. Despite the very challenging problem of instability with N-donor complexes of gold(I), several pure isomers of (phenylpyridine)gold(I) chloride have been prepared. Unfortunately, the 2-phenylpyridine isomer was never isolated in pure form, and so this remains a challenge for the future. (2-Ferrocenylpyridine)gold(I) chloride was also not isolated, despite the relative of stability of the 3-isomer compared to the phenylpyridines. This remains largely a function of the small amount of ferrocenylpyridine available and the consequent small scale on which experiments were conducted. In future, it may be possible to prepared larger quantities of all ferrocenylpyridine isomers via reaction of lithiated ferrocene with a large excess of pyridine (244) All three isomers of (phenylpyridine)(pentafluorophenyl)gold(I) have also been prepared in moderate yield and good purity. The yields for these complexes could most likely be improved by simple alterations to preparative methodology (see discussion, above)

Stable phenylpyridine complexes of gold(III) have been prepared via a simple method. The equivalent ferrocenylpyridine complexes remain something of a puzzle, however. These compounds are extremely unstable, greatly limiting the scope for characterisation. Nevertheless, the data that have been acquired suggest that complexation induces the formation of a chloro-bridged gold dimer. If this hypothesis is confirmed, this complex would appear to represent the first-ever dimer of this sort containing a neutral organic ligand. The 2-ferrocenylpyridine aurocycle proposed above (Figure 26) is likely to remain

unattainable via the techniques used for the equivalent 2-phenylpyridine aurocycle, since the relevant intermediate is so unstable. However, the same target may be attained via reaction of lithiated 2-ferrrocenylpyridine (245) directly with solid tetrachloroaurate ion. This should therefore be an aim of future work.

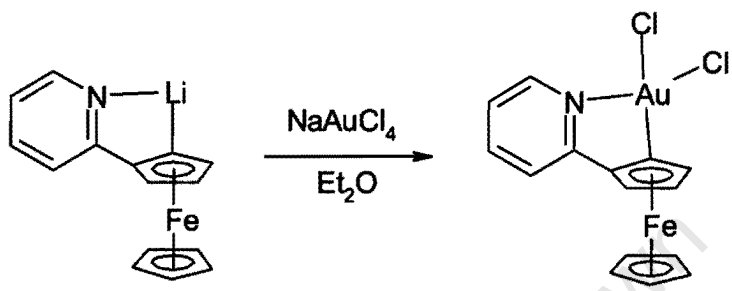


Figure 56. Possible route for preparation of C,N-donor 2-ferrocenylpyridine aurocycle

Several derivatives of the μ-chloro(1,5-cyclooctadiene)rhodium(I) dimer containing pyridyl ligands have been prepared, via established methods. These compounds were found to be generally stable in air in the solid state and in solution, although their decomposition in DMSO has been described. Although the analogous starting material, chlorobis(cyclooctene)rhodium(I), has been prepared successfully, pure pyridine-based complexes derived from this have been elusive. This may be accounted for by the greater lability of the starting material, possibly leading to displacement of the cyclooctene ligands. More successful syntheses may be achieved in future by greater control over reaction conditions, including stochiometry and time of reaction.

Although the decomposition product of **22** that has been isolated is interesting, not least in respect of its biological activity, it seems safe to conclude that the decomposition process has not been fully characterised. The identity of the initial decomposition product, corresponding to the ^{1}H NMR signals appearing after a relatively short period of solution in DMSO, remains unknown. As such, it

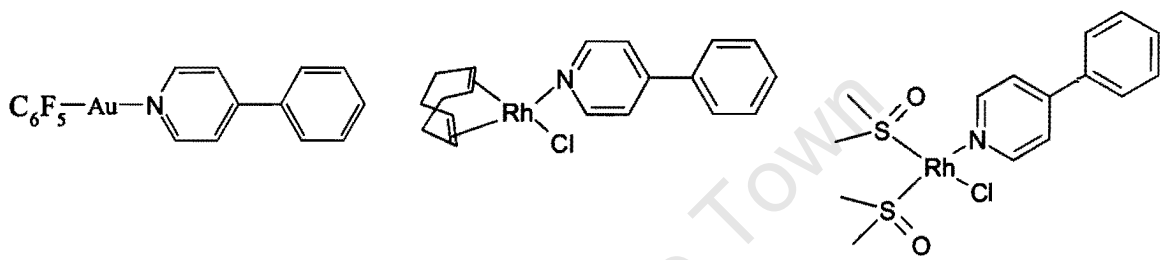
is unclear which product or products exert the major biological effect, both with respect to cytotoxicity and DNA binding. The use of a "standard solution" that may be altering in chemical composition between experiments is clearly less than ideal, as it leads to experimental inconsistency. Indeed, it is very possibly this effect that resulted in the lack of reproducibility across gel shift experiments (and in contrast to the consistency of the cisplatin positive control).

It seems fair to conclude that the experimental methodology attained in producing the active rhodium compound is seriously flawed from the view of reproducibility. In future work, it would be profitable, firstly, to make better use of more labile cyclooctene complexes of rhodium(I) so that reactions with DMSO (and other ligands) would occur on a more manageable time scale. The products of these reactions might then be better controlled by standard techniques, such as stochiometry, temperature and reaction time. Indeed, the aim of this would be to prepare the active compound(s) by a series planned *reactions*, rather than as the result of poorly controlled decomposition.

Biology

Several complexes of gold(I), gold(III) and rhodium(I) have been assessed for biological activity. Of the gold(I) compounds prepared, those containing chloride counter-anions are too unstable in DMSO and (presumably) physiological medium to be effectively tested for biological efficacy. In contrast, those with a pentafluorophenyl counter-anion are sufficiently stable in solution to allow for cytotoxicity testing. Indeed, of the complexes investigated here, compound **8** was found to have the lowest EC_{50}. Although time limitations disallowed a detailed study of this compound and its isomers prepared here, it might very well be profitable in future to carry out more detailed biological characterisation of these compounds. This should include EC_{50} studies including a lower range of concentrations, so as to enable better resolution of the correct value. Also quite

interesting would be certain mechanistic studies, as both the different chemistry and the much lower EC_{50} indicate that this compound exerts activity via a different mechanism to cisplatin. Phosphine derivatives of gold(I) are thought to exert activity via mitochondrial pathways (123), so various assays on mitochondrial function would be a good starting point, if only to distinguish this compound from these other complexes of gold(I).



8 **22** **27**

Figure 57. Biologically important compounds investigated in this study. An extremely active compound of gold(I) (**8**); an inactive compound of rhodium(I) (**22**), and a proposed structure for its more active breakdown product (**27**).

Preliminary screening revealed that none of a representative sample of the gold(III) compounds prepared showed significant cytotoxic activity. Moreover, despite previously reported results showing promising activity (88), none of the rhodium(I) complexes tested were found to be active in screening assays. It was hypothesised, and then confirmed, that this discrepancy is due to the breakdown of these complexes in DMSO. A representative compound (**22**) was selected for further study. It was determined that the activity of its breakdown product (**27**), in both "raw" and purified form, was comparable to that of cisplatin in the WHCO1 cell line. Interestingly, a variant of this cell line only slightly resistant to cisplatin was found to be extremely resistant to this breakdown product. This cross-

resistance suggests that this product exerts cytotoxicity via mechanisms similar to cisplatin.

Since cisplatin is known to exert activity via binding to DNA, it was decided to determine whether this active product also underwent these sorts of interactions. Some interaction with DNA was indicated by the results of gel shift experiments. These results, however, were not sufficiently reproducible to draw any conclusions. The more sophisticated circular dichroism protocol, however, did confirm that some interaction was taking place. In future work, this protocol could be improved in several ways to yield more informative results. Firstly, effort should be made to eliminate DMSO from the buffer mixture, as this has its own effect on the conformation of DNA. Thus, while the compounds tested do show genuine interaction with DNA, the nature of this interaction might be substantially different to that which takes place under normal physiological conditions. Secondly, these experiments should be repeated over a broader range of concentrations, so as to show progressive alterations in DNA conformation as more compound binds. Thirdly, some effort should be made to find a time scale on which binding can be observed progressively (i.e. before saturation), allowing for some understanding of the kinetics of interaction.

There are several other assays that could usefully applied to elucidating the mechanism of action of the cytotoxic gold(I) and rhodium(I) compounds investigated here. One is the flow cytometric cell cycle assay described in Chapter 1, for ascertaining the effects of drugs on the cell cycle. This would other further comparisons between these compounds and cisplatin, since the latter has been characterised extensively by this technique. The cell cycle assay gives some idea of the proportion of cells undergoing apoptosis, in terms of the size of the sub-G1 population. More direct assays, however, detect the apoptotic protease caspase-3 directly, by making use of fluorogenic substrates of this enzyme. A test of this sort would help determine by what route of cell death

these compounds are exerting cytotoxic effect. Finally, it may be useful to determine what signalling pathways can be implicated in initiating cell death. Since many cancer cell lines are p53 negative (double-mutant), it should be possible to compare the efficacy of these compounds in matched p53-positive or negative cell lines. This will help ascertain whether the mechanism of cell death is p53-dependent or independent. Conversely, protein-blotting experiments would help ascertain the effect of compound treatment on growth signalling molecules such as JNK, NF-Kβ and AKT.

Chapter 5. References

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